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Research Article

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Active Antibiotics Production by Actinomycetes Indigenous To Saudi Arabia Soils

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Abstract: *Streptomyces* are the most popular among the *Actinomycetes* groups and found in soils worldwide. They form an important part of the soil ecology within the *Actinomycetales* order. *Streptomyces* are diverse as secondary antibiotic metabolites such as Novobiocin, Amphotericin, Vancomycin, Neomycin, Gentamicin, Chloramphenicol, Tetracycline, Erythromycin and Nystatin. Thus, the current study was aimed to isolate, identify and assess the active antibiotic metabolites produced by different actinomyces sp. found in Saudi Arabian soils. Six samples were collected from desert soils of the Al Thumamah area and analyzed using GS-MS. Scanning Electron Microscopy was used to identify the bacterial strains along with their antibiotic metabolites effectiveness of secondary metabolites (antibiotics) against different Gram-positive (*Bacillus subtilis, Staphylococcus aureus*), negative pathogens (*Pseudomonas aeruginosa, Escherichia coli, Salmonella suis*, and *Shigella sonnei*) as well as the fungal strain *Candida albicans* was investigated. Thirty active bacterial (F1-30) strains were isolated from the soil samples and the strains F3, F7, F22, F30 have white, gray, pink, yellow and red colours respectively. Only ten strains (F13, F14, F15, F16, FI7, F18, F19, F20, F21, and F22) were found to have antimicrobial activity against at least one pathogen. The optimum growth environment was pH 4-10, temperature (30^0C), and NaCl (7% w/v) concentration. According to our findings, the extreme desert environment of Al Thumamah from Saudi Arabia is rich in its actinobacterial population with diverse colouring groups and various physiological and biochemical properties. This shows it's capability of generating secondary metabolite elements that could inhibit pathogenic microorganisms.

Keywords: *Natural Antibiotics; Actinomycetes; Saudi Arabia Soil; Atinobacteria Strains; Bacterial Isolates*

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1. INTRODUCTION

Actinobacteria is one of the dominant bacterial phyla and *Streptomyces* are one of the largest bacterial genera. *Streptomyces*[1], is also known as Gram-positive bacteria with high guanine (G) and cytosine (C) elements in their DNA[2]. [3] This bacterium includes freshwater as well as marine and a common soil life, which plays an essential role in the decomposition of organic materials such as chitin and cellulose. Thus, they present a fundamental element in the carbon cycle and turnover of organic matter in natural settings. They also play significant roles in the sustainability of the ecosystem by degrading lingocellulosic plant residues and recycling the nutrients back into the environment.[4] Also, they serve as an important biological control agents in the management of fungal diseases and the enhancement of plant growth.[5] Some create branching filaments that resemble the fungi mycelia where they are classified originally as actinomycetes. Some of them are aerobic while a few such as *Actinomyces israelii* could thrive under anaerobic conditions. The term "*Actinomycetes*" is derived from the Greek "atkins" (a ray) and "mykes' (fungus) which have the characteristics of both fungi and bacteria.[6] Some species of actinomycetes provide external spores; as they are responsible for the particular odour generated after rain from the soil which is called geosmin.[7] *Actinomycetes* are filamentous, sporulating colonies and recognized as a transition group between primitive bacteria and fungi. [8] Actinomycetes are also known as slow-growing bacteria and micro goldmine having useful secondary metabolites. [9] The most valuable secondary metabolites that act as antibiotics like Novobiocin, Amphotericin, Vancomycin, Neomycin, Gentamicin, Chloramphenicol, Tetracycline, Erythromycin, Nystatin, etc are produced by actinomycetes. [9] *Streptomyces* are the most popular among the *Actinomycetes* groups, and found in soils worldwide that form an important part in the soil ecology within the *Actinomycetales* order. *Streptomyces* are metabolically diverse and can utilize almost anything as a carbon source due to their ability to produce extracellular hydrolytic enzymes including sugars, alcohols, amino acids, organic acids, aromatic compounds, and other complex substrates such as cellulose, mannan, and xylan. They are also well known for their ability to produce antibiotics and other secondary metabolites having antifungal, antibacterial, antitumor, antiparasitic, immunosuppressive, and anticancer activity. [10] Thus, these microorganisms have been implicated in the antagonism and used as potential biological disease control agents against a wide variety of plant pathogenic bacteria, fungi and nematodes. [4] Cruz *et al*.,'s (2017) [11] study investigated the glycolipid depsipeptide Ramoplanin antibiotics from Actinomycetes spp, and suggested the presence of new ramoplanin analogs compound among various spp of Actinomycete producers. Antimicrobial compounds were isolated by Charousova et al (2017) from the actinomycetes spp obtained from coastal island soil samples.[4]The researchers used the spread plate technique for isolating the actinomycetes, based on multiple dilution processes on starch casein agar. The results showed that 63% of isolates have antibacterial activity, and 16% have antibiotics activity (both antibacterial and antifungal activity). The isolates include Streptomyces scabrisporus, Streptomycess parsogenes, Streptomyces misakiensis, Streptomyces cirratus, Streptomyces lincolnensis, Streptomyces endophyticus, Streptomyces chartreusis, and Streptomyces alboniger. Ouchari *et al*., (2019) [13] isolated the actinomycetes spp from Merzouga desert to investigate the antimicrobial production of these actinomycetes isolates and the results showed that 59% of isolates have antibacterial activity against one or more Gram-positive and/or Gram-negative microorganisms.Finally, the local study of Al-Dawalibi (2019)[14] isolated the active actinomyces from western region of Saudi Arabia and characterized by the production of antimicrobial agents. The researchers were successful in isolating Streptomyces , showing effectiveness against drug-resistant bacterial strains without any toxicity.

2. MATERIALS AND METHODS

1.1 Study area and collection of samples

A total of six soil samples was collected from Al Thumamah area of Saudi Arabia. Samples were collected from a depth of 15cm after removing nearly 3 cm from the unclear surface. The collected samples were positioned in sterile polythene bags and tightly closed to avoid any contamination. Subsequently, after categorization, the samples were dried at 25 °C and stored in sterile plastic containers until needed for a week.[15]

2.1 Actinobacteria isolation

To isolate the actinobacteria from samples, the serial dilution technique was implemented.[16] One gram of air-dried soil was suspended in a tube with 10ml sterile distilled water, stirred and capped with a vortex mixer (VTX-3000L) for one minute.[16] 1ml suspension of the soil was diluted serially to present dilutions of 10^{-1}, $10^{-2,}$ and 10^{-3} in sterile distilled water (9ml). Tap Water Agar (TWA) media was prepared with 1L tap water and 22g agar (WINLAB, UK).[17] Aliquots (0.1ml) of each of the three final dilutions were transferred to Petri dishes containing the isolation medium without any antifungal or antibacterial elements in and spread over the surface using a sterile glass spreader.[17] After that, the plates were incubated at 30 °C for 2 weeks.[15]

3.1 Colony enumeration

The colonies from every sample were determined after incubation using the colony count technique.[18] and the resultant colony count is shown in Table 1.

4.1 Determination of colour group

The selected isolates were cultured on Oatmeal Agar (OA) media and refined on Yeast Starch Agar (YSA) for the determination of the colour group.[19] The OA media included 15 g agar (WINLAB, UK), 30g oatmeal (Quaker), and 1L distilled water.All the gathered plates were incubated for 1 week at 30 °C. The colours of the colony within the selected isolates[15] were determined visually after the period of incubation, using the colour chart of ISCC-NBS.[20]

5.1 Actinobacterial cultivation for the production of secondary metabolites

Each isolate of actinobacteria containing 100 mL of Starch Glucose Yeast (SGY) broth was inoculated into a 250 ml flask. (Every litre of the broth contained the following): [21]

- 10g glucose (Fluka, UK).

- 10g starch soluble (Avonchem, UK).
- 2.5g cornflower (Riyadh food).
- 10g glycerol (Winlab, UK).
- 2g yeast extract (Winlab, UK).
- 5g peptone (Biochemical, UK).
- 1L distilled water.
- 3g $CaCo_3$ (Winlab, UK).

6.1 Secondary extraction of metabolites

The secondary metabolites were obtained from the broth by solvent extraction method.[22] The medium including the growing isolate also included 1:1 v/v methanol (Panreac, E.U).[22] The flasks were placed in the shaker for 3 days after filtering the contents to separate the liquid from mycelium.[22] Then, the filtrate was taken to a hot air oven to vaporize the methanol to dryness. From this, two distilled water drops were added to the remaining and the eventual crude extract was examined for antimicrobial activity.[22]

7.1 The activity test of antimicrobial

Pseudomonas aeruginosa ATCC 27583, *Escherichia coli* ATCC 2592, *Salmonella suis* ATCC 13076, and *Shigella sonnei* ATCC 11060 gram-negative bacteria were used by for the test; while the Gram-positive bacteria used were *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 13076 and one fungal strain *Candida albicans* ATCC 10231. The agar well diffusion method was implemented to analyze the antimicrobial activity.[23] Wells were filled with 0.5 mL extract. Each test organism was inoculated on to surface of the Mueller-Hinton agar medium (MHA).[23] The plates werethen incubated for 24 h at 37°C, after which the inhibition zones were measured.

8.1 Gas chromatography-mass spectrometry (GC-MS) analysis

Relying on the antibacterial activity, 10 isolate extracts were chosen for GC-MS analysis to identify the antimicrobial element.[24] The analysis was performed using the National Institute Standard and Technology (NIST) database, comparing the unknown elements with the spectrum of the known elements in the NIST database.[15] About 0.1 mL of every extract was combined with 1 mL HPLC grade ethanol (WINLAB, UK) and filtered with a 0.22 µm millipore filter to gain a crystal clear sample. Element's identification was conducted by mixing an RT x -5 columns (30 × 0.32 nm) of GC-MS model with a crystal clear sample of 1 µl (Perkin Elmer, Clarus 500, USA); while helium (3 ml/min) was implemented as a bearer gas. The following were used as a temperature gradient program - 75°C for 2 min at the rate of 50°C per min and an increase from 75 - 175°C for 7 min. The mass-to-charge ratio (m/z) peaks representing the characteristics of the antimicrobial fractions were compared with the MS database of the corresponding organic elements.[25] The extract's elements were tested in King Saud University's central laboratory at Riyadh. Identification of the extract's chemical constituents was conducted by a Perkin Elmer gas chromatograph (Clarus 500, USA) with MS (Clarus 500, USA). Furthermore, the molecular weight of the substances were confirmed using MeOH as CI ionizing by GC/MS.

9.1 The morphological characterization of the isolates using scanning electron microscopy (SEM)

The SEM (JEOL, JSM, 3060) was used to prepare and test the bacterial cells at the Central Laboratory of King Saud University according to the initial dehydration and fixation steps suggested by Moore *et al.* [26] Bacterial cells were placed at 24 °C for 24 h with 3% glutaraldehyde and washed 3 times with distilled water for 10 minutes each and placed with 1% Osmium tetroxide overnight.[26] The dehydration was done with serial ascending ethanol concentrations of 30 – 50 – 60 – 70 – 80 – 90 - 100% for 10 min each and with 100% overnight.[26] Finally, the cells were dried with a critical point dryer; where these samples were coated in gold using a gold sputter and analyzed using the SEM.[27]

10.1 Physiological features of the isolates

The Physiological features of the isolates where determined over a increasing pH range of 4-10 and 10% NaCl , following the growth at several temperatures of 30, 40, and 50°C were also determined using YSA media.[28-31]

11.1 Biochemical features of the isolates

The isolates were analyzed for urease production, gelatin hydrolysis[31], casein hydrolysis [32,33], catalase production[34], the utilization of citrate and carbon sources like lactose and glucose, as well as the production of H_2S.[29] All the characteristics of the selected isolate were determined after 7 days of incubation at 30°C using YSA. The aerial mycelium colour, growth, and diffusible pigmentation were observed after incubation.

12.1 Analysis of the cell wall

The isomers of Diaminopimelic Acid (DAP) are essential elements in Gram-positive bacteria and its cell wall peptidoglycan such as actinobacteria.[35] If a Gram-positive bacterium includes peptidoglycan with one isomer of DAP, the DAP would be placed in the wall of the cell; thus, peptidoglycan's DAP isomers could be determined by analyzing the whole cell.[35] To accomplish the hydrolysis of bacterial cells, 50 µl of 6N HCl (Winlab, UK) and nearly 3 mg of dried cells were hydrolyzed in an Eppendorf tube. After centrifugation, it was autoclaved at 121°C for about 15min. After this,the supernatant was examined by plates of Thin Layer Chromatography (TLC).[36] Every samples included 3 µl of the baseline celluloses within the TLC plate using a capillary tube (20 cm X 20 cm) (Merck No.126827). 10 mg of 1µl (0.01g) diaminopimelic acid (DL)-2, 6-DL (DAP) was also included. These elements are a combination of the isomers within DAP. TLC was analyzed with a system including a covered tank of methanol-water-6N HCl-pyridine (80:26:4:10 v/v), for nearly 4h. Spraying 0.2% ninhydrin solution which includes 0.2g ninhydrin, the spots were visualized with 100ml acetone and a temperature of 100 °C for 10min. The isomers showed violet colour with 0.24 (meso- and DD-isomer) and 0.29 RF (LL-isomer).[37,38]

13.1 RESULTS

14.1 Actinobacteria isolation on WA

After the process, 30 colored actinobacteria isolates were obtained as a result of TWA isolation[39]; where only 10 isolates were selected for further research.

15.1 Isolated colonies

Table 1. The colony count obtained from the third dilution of all soil samples

Sample's origin	Dilutions		
	10^{-1}	10^{-2}	10^{-3}
	Colonies		
Thummah 1	Colonies more than (300)	145 colonies	Very few colonies to count (less than 30 colonies)
Thummah 2		151 colonies	
Thummah 3		192 colonies	
Thummah 4		200 colonies	
Thummah 5		164 colonies	
Thummah 6		155 colonies	

Table(1) shows the colony count obtained from the third dilution of all soil samples[18].

Table 2.* The CFUs per ml for each soil sample collected *from Al Thumamah region in Saudi Arabia.

Sample	Colonies	CFUs
Thumamah 1	145 colonies	1450X102 CFUs/ml
Thumamah 2	151 colonies	1510X102 CFUs/ml
Thumamah 3	192 colonies	1920X102 CFUs/ml
Thumamah 4	200 colonies	2000X 102 CFUs/ml
Thumamah 5	164 colonies	1640X102 CFUs/ml
Thumamah 6	155 colonies	1550X102 CFUs/ml

Table (2) presents the CFUs per ml collected from the second dilution of the environmental samples. [15].

16.1 Colour grouping

10 strains were cultured in OA medium and the colour strain of each strain determined was shown in the following table.

***Table 3.* The color groups presented in the strains, *Biased on the color of their mycelium* the ten strains grouped into specific color groups**

NO	Name	Growth Rate	Color Group	Pigmentation
1	F13	+++	Light gray	-
2	F14	+++	Whitish gray	-
3	F15	+++	Whitish gray	-
4	F16	+++	Whitish yellow	-
5	F17	+++	Grayish creamy	-
6	F18	+++	Light gray	-
7	F19	+++	Dark gray	-
8	F20	+++	Beige	-
9	F21	+++	Yellowish white	-
10	F22	+++	Brownish light gray	Brown

+ - indicates the degree of growth , - indicates absence of pigmentation

17.1 Antimicrobial activity of the isolate extracts

All the 30 isolated actinobacteria were examined for any activity against the 7 pathogenic microorganisms, *S. sonnei* ATCC 11060, *S. aureus* ATCC 13076, *B. subtilis* ATCC 6633, *P. aeruginosa* ATCC 27583, *E. coli* ATCC 2592, *C. Albicans* ATCC 1 0231, and *S. suis* ATCC 13076[23,40,41]. From these 30 strains, only ten exhibited the required activity against at least one pathogen. labeled as F 13, F 14, F 15, F 16, F 17, F 18, F 19, F 20, F 21, and F 22 respectively. The ' antimicrobial activity of the strains is shown in the following table-4.

Table 4. Measurement of the inhibition zone in milliliters produced by the extracts

Extract Code	Pathogenic microorganism						
	Gram-negative bacteria			Gram-positive bacteria		yeast	Yeast
	(9)	(10)	(11)	(12)	(13)	(14)	(15)
	S. suis	*E. coli*	*S. sonnei*	*P.aeruginosa*	*B.subtilis*	*S.aureus*	*C.albicans*
	ATCC 13076	ATCC 2592	ATCC 11060	ATCC 27583	ATCC 6633	ATCC 13076	ATCC 10231
F13	-	10mm	15mm	-	11mm	-	13mm
F14	-	-	-	8mm	10mm	-	-
F15	10mm	-	-	-	13mm	-	10mm
F16	-	14mm	12mm	-	12mm	20mm	-
F17	-	-	13mm	10mm	9mm	8mm	15mm
F18	11mm	11mm	18mm	13mm	10mm	-	15mm
F19	-	-	-	-	-	7mm	-
F20	-	-	9mm	-	-	6mm	-
F21	6mm	-	14mm	-	9mm	-	13mm
F22	9mm	-	10mm	14mm	14mm	8mm	-

18.1 Scanning Electron Microscopy

SEM analysis showed that all the 10 isolates presented various morphologies.. The highly branched and coenocytes of Arial mycelium were generated and the spores were shown to be spiny, smooth, or wart-like[42,43] (Figure 1).

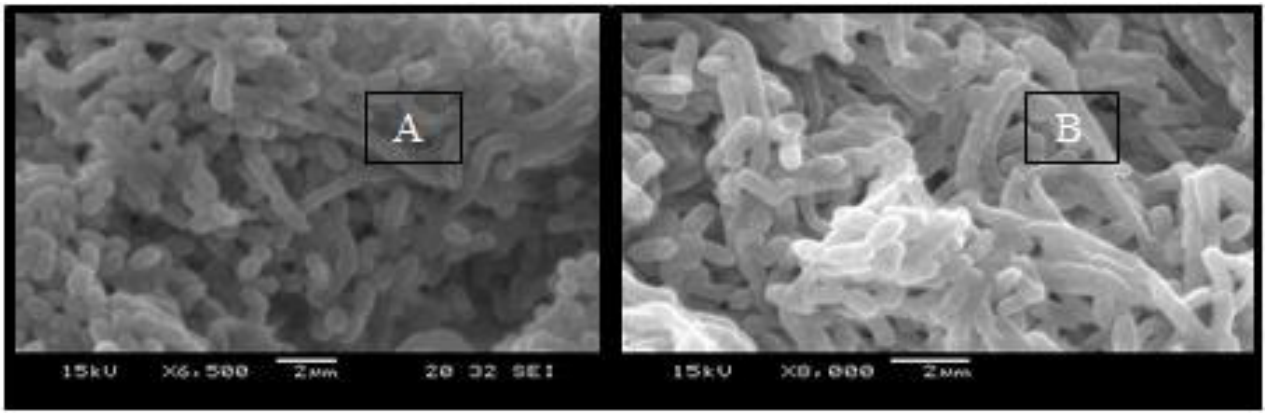


Fig 1- SEM analysis for the (A) F13 and (B) F14 revealing spore-forming *Streptomyces.*

19.1 GC – MS Analysis

The GC-MS analysis was used to test the extracts; where a significant number of elements were identified for every isolate[25]. Some have shown antibacterial activity and other elements were shown in all extracts at different concentrations which were seen in a few[26]. The identified elements and their appearances in the 10 isolates are presented in the table -5.

Table 5. GC-MS analysis showing the elements detected in the Actinomycete extracts.

	Element	M.W	Formula	Percentage of Similarity
1	2,3-Butanediol, [R-(R*,R*)]-	90]	C4H10O2	99
2	Cyclobutanol	268	C4H8O	71
3	Octadecanal	72	C18H36O	63
4	Oleyl alcohol	282	C18H36O	42
5	Oleic acid	268	C18H34O2	25
6	Eicosanoic acid	312	C20H40O2	25
7	Acetic acid	338	C2H4O2	24
8	Heptanal	92	C7H14O	15
9	Glycerin	114	C3H8O3	10
10	Erucic acid	60	C22H42O2	6

20.1 Biochemical and Physiological Features of the Isolates

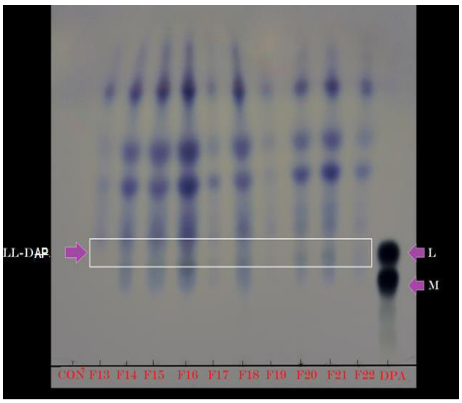
The isolates grew well at pH 4 - 10, 30 - 45°C, and 7% NaCl concentrations. [44-,46]. Nearly all strains of coagulated milk and hydrolyzed gelatin presented the ability to bring urea hydrolysis[31], peptonization of milk, and use citrate implementation[16]. Some showed glucose fermentation, while others showed lactose; however, three isolates produced H_2S as shown in the following table[29].

Table 6. The biochemical and physiological features of the 10 isolates.

Strain No	Temp °C			pH			NaCl Conc.			Gelatin hydrolysis	Catalase	Casein hydrolysis		Urea hydrolysis	Citrate Utilization	Sugar fermentation		H_2S production
	30	45	50	4	7	10	1	7	10			Coagulation of milk	Milk peptonization			Glucose	Lactose	
F13	+	+	-	+	+	+	+	-	-	+	+	+	+	+	+	-	-	-
F14	+	+	+	-	+	+	+	+	+	-	+	+	+	+	-	-	-	-
F15	+	+	+	-	+	+	+	-	-	+	+	+	+	+	-	-	-	-
F16	+	+	+	-	+	+	+	+	-	-	+	+	+	+	+	-	-	-
F17	+	+	+	-	+	+	+	-	-	+	+	+	+	+	-	+	+	+
F18	+	+	-	-	+	+	+	+	-	+	+	+	+	+	-	+	+	-
F19	+	+	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	-
F20	+	+	-	-	+	+	+	+	-	+	+	+	+	+	-	+	+	-
F21	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	-	-	-
F22	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-

21.1 Cell Wall Structure analysis

DAP isomers in the hydrolysates of the cell wall within the examined strains were set by the TLC following Boone and Pine (1968)[47] and Waksman and Henrici (1943)[48] as per the standard approach (Figure 2).



Strains: LL-DAP (L) and meso-DAP (M) , control (C).

Fig 2- Thin layer chromatography analysis. Photographs showing whole cell diaminopimelic acid(DAP)of some Streptomyces

3. DISCUSSION

Streptomyces strains are widespread in soils.[49,50] Their ability to produce a wide range of extracellular enzymes and valuable bioactive secondary metabolites is of use in development of industries, pharmacy and agriculture.[15] Actinobacteria produce pathogenic fungal cell wall degrading enzymes, a range of siderophores, toxic hydrogen cyanide (HCN), and antimicrobial compounds.[51-53] As a result, they are capable of showing direct and indirect effects on microorganisms in the soil. [51-53]In this study, 30 Actinobacteria isolates were obtained from soils of Riyadh desert using a TWA medium. A broad range of media has been implemented to isolate the bacteria genus members, including starch-nitrate agar[54] and starch casein agar.[55,23] TWA media was used in the study which showed useful for the isolation of many Actinobacteria numbers as reported by Ara *et al.*[39] Many studies on ' antimicrobial activity of the Actinobacteria have been conducted.[40,41] In the current study, 30 isolates were analyzed for their ability to generate antimicrobial metabolites; however, just 10 isolates were selected for further research. These selected isolates manifested antimicrobial activity against at least 1 out of 7 human test pathogens including *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 13076, *Escherichia coli* ATCC 2592, *Shigella sonnei* ATCC 11060, *Salmonella suis* ATCC 13076, *Pseudomonas aeruginosa* ATCC 27583, and *Candida albicans* ATCC 10231. From this, 45% of the isolates manifested antimicrobial activity with the *Salmonella suis* ATCC 13076, 35% manifested reasonable activity against *Escherichia coli* ATCC 2592, 55% of the strains manifested marked activity against *Pseudomonas aeruginosa* ATCC 27583,

55% inhibited *Shigella sonnei* ATCC 11060 pathogen, 80% were quite efficient against *Bacillus subtilis* ATCC 6633, 55% inhibited *Candida albicans* growth ATCC 10231, and 50% inhibited the *Staphylococcus aureus* ATCC 13076. SEM research showed that all selected isolates manifested aerial mycelium, which was significantly branched and coenocytic.[42,43] Spore forms varied between spiny, smooth, and wart-like in certain chains; where the isolates were *Streptomyces* species[42,43]. Many groups of different colours divided into gray, white, yellow, brown, pink, creamy, and red were manifested among the isolates. The groupings were based on this standard. Such a diversity of coloration in the colony shows that the extreme weather environment in the Kingdom's desert presents a broad range of groupings in colour amongst the Actinobacteria. The strains were categorized taxonomically using genotypic and phenotypic measurements. The emergence of good growth rates were seen on both OA and YSA medium. Varieties of colours were present, which included whitish-gray in two strains coded as F14 and F15, light gray in only two strains which were F13 and F18, yellowish-white in the strain F21, beige in the strain F20, whitish-yellow in the strain F16, dark gray in the strain F19, creamy in the strain F17 and brownish-gray in F22. Pigmentation was defused by only one strain, F22 out of the ten . The work of Lo *et al*.,[56]and Mohamed *et al*., [57] stated that the majority of the Actinobacteria soil were white, brown, or gray with many being gray and white. All of the tested isolates could grow up to 7% without reaching NaCl of 10%, making them some what halo tolerant.[44,45] Nevertheless, 3 isolates did not grow on the identified concentration and were not regarded as halo tolerant *Actinobacteria*. Normally, Actinobacteria are considered thermo tolerant and could grow up to 50°C.[46] In the thermal test, it appeared that most strains could grow at 45 ° C, but no strain grew at a temperature of 50°C except the strains: F14, F15, F16, and F17. The Actinobacteria soil could liquefy gelatinase presented in the results and only 2 isolates were incapable of solubilization. Furthermore, all analyzed isolates showed the ability to produce catalase which are known features of such bacteria; thus, confirming the results of Ajay *et al*.,[34,41] Peptonization and hydrolysis of the milk protein casein is a result of the caseinase enzyme activity which is a protease enzyme that breaks the long chain of casein protein in the milk to peptides. The strains that were positive to this test could produce the enzyme and peptonize the milk. In the test for urease production, all strains showed a positive result in the urea broth, a result that confirms the work of Ismet *et al*.[17] In this analysis, 4 isolates utilized citrate; where a similar study found that several isolated Actinobacteria from the Kingdom's soil can utilize citrate as a source of carbon in the lack of sugar.[17] About 40% of all the 10 selected strains, indicated by the yellow tube, had fermented both lactose and glucose and the remaining 60% could not ferment lactose or glucose; yet, utilized amino acids as a source of nutrient in the medium. Only 10% were able to generate H_2S, which was shown by the formation of black color in the medium. Bacteria showed normal growth when offered DAP being an amino acid; while in DAP's absence, they did not generate new peptidoglycan within the cell wall. DAP exists in 3 stereoisomeric types, which are the DD, LL, and meso-isomers.[58] The *Streptomyces* genus is featured by the LL- DAP in the cell wall, revealing that the 10 strains included the LL-DAP as a diagnostic acid. The current findings showed that the cell-wall peptidoglycan was seen in all isolates, therefore confirming that they are *Streptomyces* species. These results are similar to the findings of Xianwen, *et al*.,[59] who analyzed the presence of LL-DAP in the entire cell hydrolysates of the ACMA006 strain.

4. CONCLUSIONS

According to the findings of this study, the extreme desert environment of Al Thumamah , Saudi Arabia is rich in its actinobacteria population with diverse colour groups and various physiological and biochemical properties. Many of these soil-inhabiting actinobacteria can generate secondary metabolite elements that could inhibit pathogenic microorganisms. These metabolites continue to be classified as one of the most important pharmaceutical remedies. Genetically, the inhibitor actinobacteria strains could possess antibiotic-producing genes that are of enormous medical significance. Further research of actinobacteria strains from extreme conditions such as the Kingdom's deserts could result in innovative findings that will facilitate the development of novel medicines against human pathogenic bacteria.

5. ACKNOWLEDGEMENT

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6. AUTHORS CONTRIBUTION

Mrs.Fetoon Alkhelaiwi conceived the practical work and wrote the manuscript. Dr. Ismet Ara and Nadin Moubayed conceived the manuscript idea. All authors discussed the methodology and results. Data available upon request

7. CONFLICT OF INTEREST

Conflict of interest declared none.

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