

Identifying Extremophilic Actinomycetes Effective Against Multidrug-Resistant Bacterial Pathogens and Demonstrating High Enzyme Activities

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Abstract

Objective: This study focused on isolating actinomycetes from an unexplored arid region of Saudi Arabia.

Methods: Actinobacteria were isolated on starch nitrate agar and identified using molecular techniques. All isolated screened for antibacterial and enzyme activities.

Results: Twenty-two different isolates were obtained on starch nitrate agar. Identification via 16S rRNA sequencing showed that 86.4% belonged to the genus *Streptomyces*, with one isolate related to *Lentzea albidocapillata*. The actinomycetes were tested for enzyme production (amylase, protease, keratinase, gelatinase, chitinase, and lipase) and antibacterial activity against various pathogens, including *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Enterococcus faecalis*, *Acinetobacter baumannii*, *Klebsiella oxytoca*, and *Escherichia coli*. Eight isolates exhibited antibacterial activity, with AL3 being effective against all tested Gram-positive bacteria and showing the largest inhibition zones. The crude extract from AL3 demonstrated strong activity against *E. faecalis*.

Conclusion: In conclusion, actinomycetes from extreme habitats can produce valuable antibiotics and enzymes, highlighting the potential for further bioprospecting in similar and unique ecological regions of Saudi Arabia.

Keywords: Actinomycetes, arid area, enzyme production, antibacterial activity

Introduction

Secondary products play significant roles in many sectors of society due to their valuable applications in industry, biotechnology, and pharmacology, particularly in treating human diseases such as cancer and bacterial infections and there is an increasing demand for microbial natural products for various industrial applications.¹ Over 22,000 microbial secondary metabolites are known, with approximately 70% are produced by actinomycetes, 20% by fungi, 7% by *Bacillus* species, and 1–2% by other bacteria.² Actinomycetes are responsible for producing nearly two-thirds of all antibiotics available on the market and their enzymes are highly valued in biotechnological industries and biomedical fields due to their stability and unique substrate specificity.³ Although many actinomycetes have been isolated and screened from soil, there remains an urgent need to discover novel secondary products to address the rising prevalence of antibiotic-resistant pathogenic bacteria and the increasing demand for more efficient enzymes in the pharmaceutical industry.³

Unexplored environments and extreme habitats are promising sources of novel actinomycetes, which are believed to be rich in interesting new compounds.⁴ Arid regions host various unexplored xerophilic, thermophilic, halophilic, and alkaliphilic actinobacteria, which produce new bioactive metabolites.⁵ Previous studies have shown that thermotolerant and thermophilic actinomycete members of genera *Streptomyces*, *Micromonospora*, *Actinomadura*, and *Streptosporangium* are more abundant than mesophilic forms in desert soil.⁶ The most widespread thermotolerant actinomycetes from desert soil produce new compounds, including ansamycin and 22-membered macrolactones, which exhibit antibacterial and antitumor activity.^{7,8} Additionally, pharmaceutically active metabolites such as ectoine

and hydroxyectoine, which had protective properties against desiccation and potential applications in industry and agriculture, have also been identified from xerophilic actinobacteria.⁵ The discovery of new bioactive compounds from unique strains of extremophilic or extremotrophic actinobacteria has raised hopes that exploring these groups could provide a new source of secondary metabolites. This research aimed to identify novel actinomycetes that produce bioactive compounds, such as antibiotics and enzymes, from an unexplored arid region of Saudi Arabia.

Materials and Methods

Samples Collecting

The samples were obtained from three different sites in an arid area (Figure 1) that was 35 km southeast of Jeddah, Bahrah Province, Saudi Arabia (21°22'34.1076"N, 39°28'47.2332"E). The soils were taken at 5–12 cm depth for the first site, 3–5 cm for uncultivated hill soil, and about 10 cm for sandy soil. The samples were collected aseptically using sterile bottles with a sterile spatula, transported at 4°C, and processed directly.

Sample Pretreatments

The soil samples were air-dried at room temperature for about 7 days before inoculation onto isolation plates. The soil suspension of each sample was made separately by mixing 1.0 g with 10 ml distilled water and vortexing for 2–5 min; then the mixtures could settle.⁹ Each sample was divided into two parts. In the first part, sample suspension was used immediately, and the other part was divided into three parts, one treated by dry heat in a hot air oven at 110°C for an hour, one mixed with 1.0 ml (1.5%, v/w) of phenol solution and one heated at 50°C for 10 min in a water bath.^{2,9}

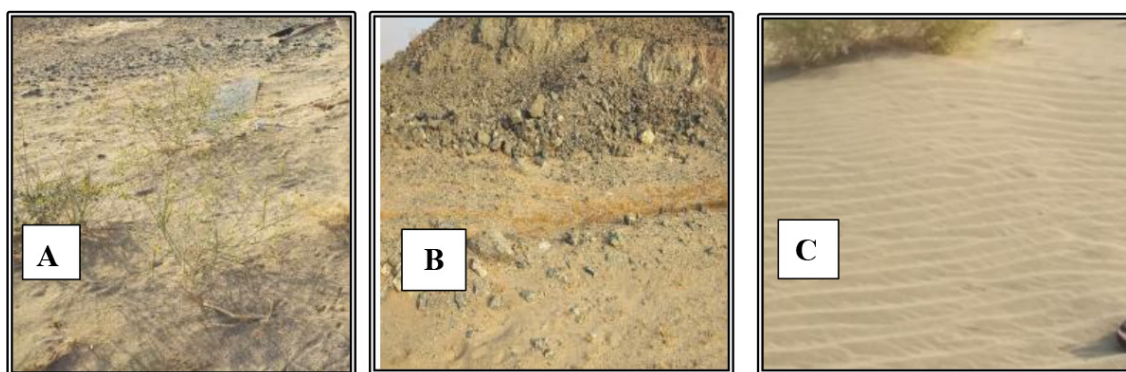


Fig. 1 The studied arid region is in Bahrah Province, Saudi Arabia. (A): cultivated soil, (B): uncultivated hill soil, and (C): sandy soil.

Isolation and Purification of Actinomycetes

As described by Al-Dhabi *et al.*,¹⁰ about 0.1 ml from each sample dilution were spread evenly with a sterile glass rod onto a starch nitrate agar medium.¹¹ The inoculated plates were incubated at 30°C for 7 days. After the incubation, morphologically distinct colonies were chosen and subcultured to obtain pure isolates. The pure isolates were maintained on starch nitrate agar slants at 4°C; for long-term storage, cultures were maintained in glycerol broth (50%, v/v) at -20°C. Taxonomic characterization, the production of some important enzymes, and antibacterial potential against some pathogenic bacteria were studied for all isolates.

Taxonomical Studies of the Selected Filamentous Bacteria

For micro-morphological characteristics, light microscope was used to observe the morphology of the isolates. The chitin agar plates were inoculated by the isolates and incubated at 30°C for 7 days to be examined by direct microscopic observation at 400x magnification. The second method was a slide culture technique with some modifications.¹² A slide culture was prepared from a 5.0 mm square block of starch nitrate agar, transferred aseptically to the center of the sterile slide, inoculated with the actinobacterial isolate, and kept in a glass Petri dish containing a wet sterile filter paper at 30°C for 3–5 days. Then, the slide was observed under a light microscope using an oil immersion lens.

Cultural and Physiological Characteristics

The growth capability, color of substrate and aerial mycelia, and presence of soluble pigments were tested using various media (ISP-2, ISP-4, ISP-7, and starch nitrate agar). The inoculated plates were incubated at 30°C for 7 days. The color of the substrate mycelium was described by the colors of the RAL code.¹³

All physiological tests were performed on starch nitrate agar medium and incubated for 7–14 days at an appropriate temperature. The growth was assayed at different temperatures (30, 35, 40, 45, 50, and 55°C) and at various pH levels (5, 6, 7, 8, and 9) to determine the optimal temperature and pH. The growth was also tested at varying NaCl concentrations, 0, 3, 6, 9, 12, and 15% w/v.¹⁰

Biochemical Characteristics

Gram staining was carried out by using the standard Gram reaction. Antibiotic susceptibility was tested on Muller-Hinton

agar (Himedia, India) plates at 30°C for 24–72 hr. with disks containing different antibiotics such as amikacin (AK), cef-tazidime (CAZ), aztreonam (ATM), piperacillin (PRL), imipenem (IMI), and ciprofloxacin (CIP). After incubation, the plates were examined for the presence of inhibition zones around the disc of the antibiotic.

Molecular Identification

The purified actinobacteria isolates were grown for approximately 5 days on a starch nitrate agar plate. Cells were collected and genomic DNA extraction was performed at King Fahd Medical Research Centre, KAU. In a clean tube, bacterial colonies were mixed with 500 µl of TE buffer. This was followed by 3 cycles of heating in a water bath at 99°C and treatment by liquid nitrogen (3 min. in each cycle). The crushed mycelium was treated with lysozyme (20 mg/ml) and incubated in a water bath at 37°C for 30 min. After cell lysis with SDS 10% (w/v) and proteinase K at 55°C for 30 min, the lysate was cooled and extracted for 5 min with a mixture of phenol: chloroform (1:1, v/v) at 10,000 rpm using a microliter centrifuge (Hettich Mikro 220R, Germany). In the aqueous phase, DNA was precipitated by adding 70–90% ethanol at -20°C for 30 min. After centrifugation, the formed pellet was washed twice with 90% ethanol and dissolved in TE buffer. To obtain RNA-free DNA, 20 µl of RNAase solution (20 µg/ml) was added and then incubated at 37°C for 1 hr. The sample was once again extracted with equal volumes of phenol and chloroform and precipitated as above. The purity and concentration of DNA were checked using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). Then, polymerase chain reaction (PCR) was used to amplify 16S rRNA using a thermal cycler (Applied Biosystems Veriti™ 96-Well Thermal Cycler, Singapore). Amplification was performed using GoTaq® Green Master Mix, 2X (Promega) and two selected primers: 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 519R (5'-GWATTACCGCGGCKGCTG-3').¹⁴ PCR conditions consisted of an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation (94°C for 40 sec), annealing (58°C for 40 sec), and extension (72°C for 1 min), and a final extension at 72°C for 10 min. The amplification product was examined by 1% agarose gel electrophoresis, and the gel was visualized by Ultra-Violet Product (UVP BioSpectrum® Imaging System, USA).

Phylogenetic Analysis of 16S rRNA Sequence

Sequencing of the PCR amplicons of the isolates was done by MacroGen, Inc. (Seoul, Korea). The 16S rRNA sequence data

were compared with available sequence data in GenBank at the National Center of Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST) program. The isolates were identified based on the sequence similarity to their closest homolog.

Screening of the Selected Isolates for their Enzyme Production

The production of extracellular enzymes was studied by inoculating on different agar media at 30°C for 7–14 days. For screening amylolytic activity, the isolates were grown on ISP-2 containing 1% soluble starch.¹⁵ After growth, the plates were flooded with an iodine solution. Amylase production was detected as a zone of clearance around the colony on a bluish-black background due to starch digestion. Iodine forms a bluish-black complex with starch but not with hydrolyzed starch.

The indicator of hydrolysis of casein was assessed by growing the isolates individually on ISP-2 containing 1% skimmed milk.¹⁵ Protease production was observed by the appearance of a clear zone around the colony in the medium.¹⁶ Regarding the screening of keratinase, the feather meal agar medium¹⁷ was inoculated with the bacterial isolates. A clearing zone around the growth indicates that this isolate has keratinase. Gelatinase production was tested using a gelatin medium containing 12% gelatin in test tubes.¹⁸ Partial or total gelatin liquefaction of the inoculated tubes even after exposure to the cold temperature of a refrigerator (4°C) was considered that these isolates produced gelatinase.¹⁹ The actinomycete cultures were further examined for their chitinase activity on plates containing a chitin agar medium. The appearance of a clear zone surrounding the colony was regarded as positive for chitinase activity.²⁰ To determine lipase activity, the isolated bacteria were streaked on a solid agar medium with Tween 80 as a substrate and the occurrence of a zone of clearance around the growth and subsequent formation of a white precipitate indicated a positive result.²¹

Screening of the Isolates for their Antibacterial Activity

The isolates were examined using the agar plug method²² against test pathogenic bacteria obtained from King Fahad Armed Forces Hospital, Jeddah. The tested bacteria were *Staphylococcus aureus* (ATCC 29213), methicillin-resistant *Staphylococcus aureus* (MRSA) (ATCC 52923), *Enterococcus faecalis* (ATCC 29212), *Acinetobacter baumannii* (ATCC BAA-747), *Klebsiella oxytoca* (ATCC 49131), and *Escherichia coli* (ATCC 25922). The isolates were spread on the starch nitrate agar medium and incubated at 30°C for 10 days for better growth and antibiotic production. Agar discs of the actinomycete isolates were removed using a sterile cork bore (5 mm in diameter) and placed onto Muller-Hinton agar (Himedia, India) plates seeded separately with cell suspensions of the test bacteria (12–24 hr. old). The plates were kept at room temperature for 2 hr. for the diffusion of antibiotic compounds from actinomycetes discs, and the plates were incubated at 37°C for 24–72 hr. Following incubation, antibacterial activity was noticed by the formation of a clear zone surrounding the agar plug. The diameter of the inhibition zone was measured in millimeters (mm), and the absence of an inhibition zone indicated a negative result. The results were expressed as a mean ± standard deviation (SD) of three replicates.

Secondary Screening of the Strain with the Highest Antibacterial Activity

The most potent antagonistic isolate was chosen and cultured in a starch nitrate broth medium and incubated at 30°C in a shaker (150 rpm) for 4 days as a seed culture. About 2.0 ml was transferred from the preculture to 50 ml starch nitrate broth and incubated at 30°C in a shaker (150 rpm) for 6 days.²³ After incubation, the cells were harvested at 4500 rpm for 20 min using the centrifuge (Thermo Fisher Scientific centrifuge, Germany), and the supernatant was collected. Antibacterial compounds were recovered by solvent extraction with ethyl acetate. Ethyl acetate was added to the supernatant in the ratio of 1:1 (v/v) and incubated in a shaker for 1 hr. for complete extraction. The organic layer that contained the active material was separated from the aqueous phase using a separation funnel. The solvent was evaporated using a vacuum rotary evaporator.²³ The resultant crude compound was re-suspended in dimethyl sulfoxide (DMSO), and then the crude extract was assayed for antibacterial potential. Secondary screening was done using the agar well diffusion method on Muller-Hinton agar (Himedia, India) plates. The agar plate was inoculated by spreading a volume of the selected pathogenic bacterium over the entire agar surface. Then, two holes with a diameter of 5 mm were punched with a sterile cork borer, and a volume (100 µl) from both crude extract and DMSO (as a control) was introduced into the wells. After incubation at 37°C for 24–48 hr., the diameter of the inhibition zone was measured in mm.

Results

Different actinomycete isolates were obtained from the arid region near Jeddah in Bahrah Province. A total of 11 isolates, labeled AL1 to AL11, were derived from untreated air-dried soil samples. Additionally, seven isolates, labeled AL12 to AL18, were obtained after treating the samples with dry heat and phenol. Furthermore, four actinomycete isolates, labeled AL19 to AL22, were isolated after pretreating the soil samples with dry and moist heat. It was observed that the highest population of actinomycetes, consisting of 11 isolates, was obtained from the soil samples without any treatment.

Cultural characteristics of the isolates on starch nitrate agar medium showed various shapes with soluble pigments for isolates AL1, AL2, AL11, AL20, and AL21. Also, it was noticed that out of 22 isolates (45.45%) had a gray color, and the remaining isolates showed different colors, such as yellow, green, brown, white, cream, blue, and pink (Figure 2).

The colony characteristics of the isolates on starch nitrate, ISP-2, ISP-4, and ISP-7 agar media showed different appearances and colors (Figure 3). All isolates showed the highest growth on starch nitrate agar, while the growth on ISP-2, ISP-4, and ISP-7 media was lower for most isolates. On ISP-2 and ISP-3, isolate AL12, AL14, and AL22 had moderate growth while AL3 and AL9 showed poor growth but AL5, AL10, and AL20 showed no growth. Melanoid pigment was detected only in isolates AL2 and AL4 (Figure 4).

Under a light microscope, all the isolates were Gram-positive with filamentous hyphae (Figure 5). The microscopic characterization of the isolate growing on chitin agar and starch nitrate agar using slide culture showed filamentous structures (Figure 6A and 6B).

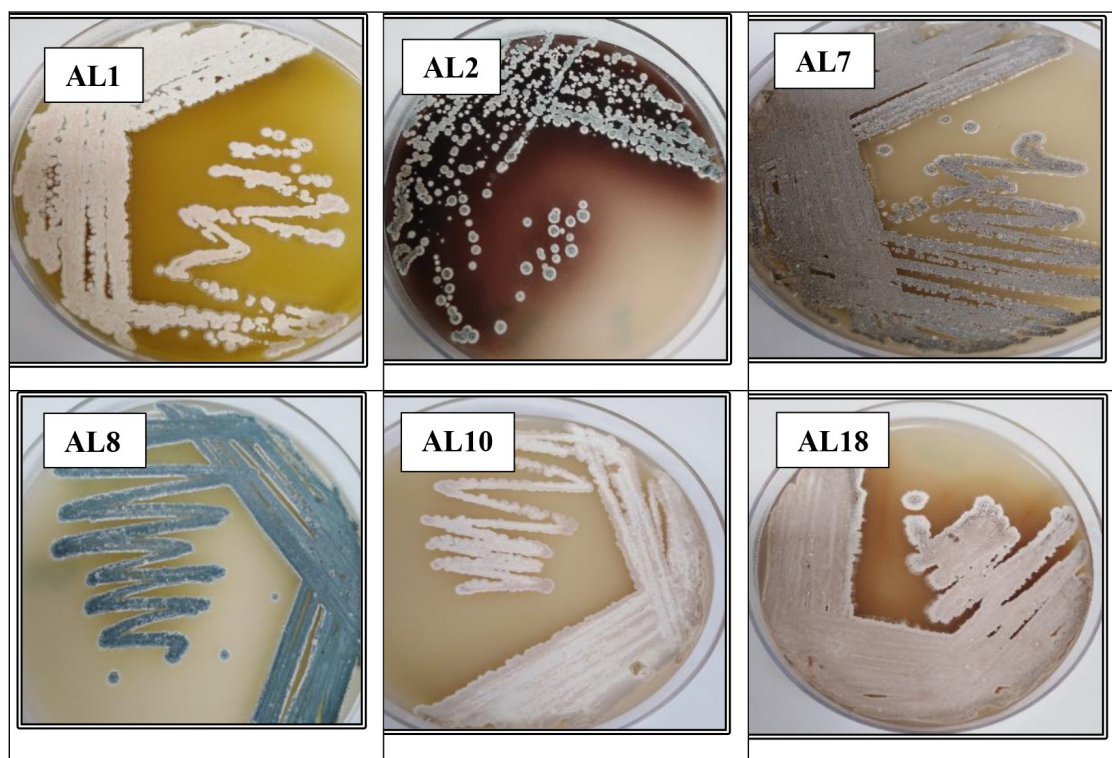


Fig. 2 Colonies morphology and color of some isolates grown on starch nitrate agar medium at 30°C for 7 days.

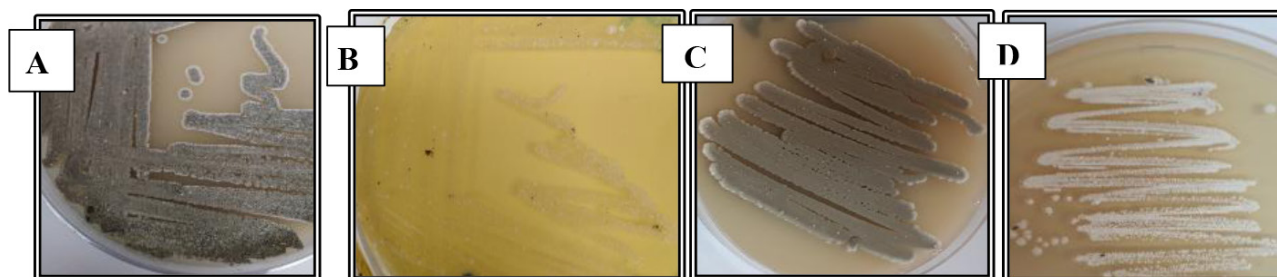


Fig. 3 Different agar media used for culture characteristics of the isolate AL14, A: Starch nitrate, B: ISP-2, C: IPS-4 and ISP-7



Fig. 4 Production of melanin pigment on tyrosine agar (ISP-7) by isolate AL4.

The results of physiological and biochemical properties revealed that out of 22 isolates, 11 could grow at approximately up to 55°C. The optimum growth temperature for all isolates was 30°C. The obtained isolates grew at a pH between 7.0 and 8.0, with the optimum pH being 7.0. Tolerance to different NaCl concentrations was varied between isolates. Four isolates, AL2, AL5, AL14, and AL18, could not grow in the presence of 3% (w/v) NaCl while the other isolates grew at 3–6% NaCl but the isolate AL19 could tolerate high NaCl concentrations up to 12% (w/v).

The growth ability of the isolates in the presence of six standard antibiotics was evaluated by the disc diffusion method. All isolates recorded a clear sensitivity (20 mm or more) against amikacin, imipenem, and ciprofloxacin; meanwhile, they demonstrated resistance (16 mm or less) against ceftazidime and aztreonam. However, about half of the bacterial isolates (AL2, AL3, AL4, AL5, AL6, AL8, AL10, AL11, AL18, AL20, and AL21) showed sensitivity profiles against piperacillin. Figure 6C shows the antibiotic susceptibility of the isolate AL2.

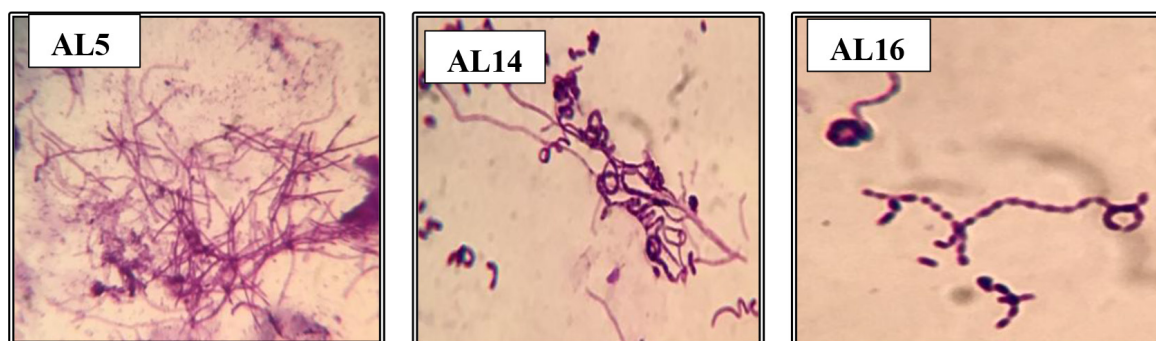


Fig. 5 Gram staining of some bacterial isolates under a light microscope (1000 × magnification).

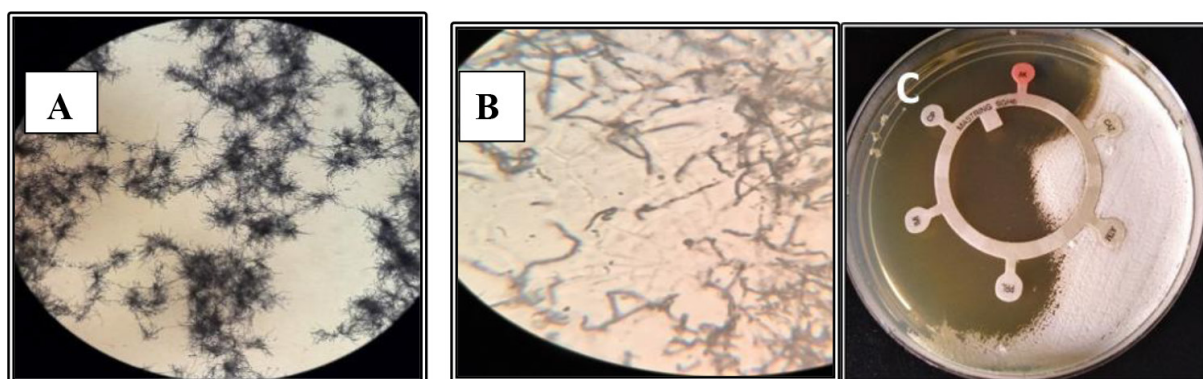


Fig. 6 Micromorphology of the isolate AL1, A: on chitin agar (400 × magnification), (B): on starch nitrate agar (1000 × magnification) using a slide culture technique, and (C): Antibiotic resistance profiles of the isolate AL2; AK: Amikacin; CAZ: Ceftazidime; ATM: Aztreonam; PRL: Piperacillin; IML: Imipenem; CIP: Ciprofloxacin.

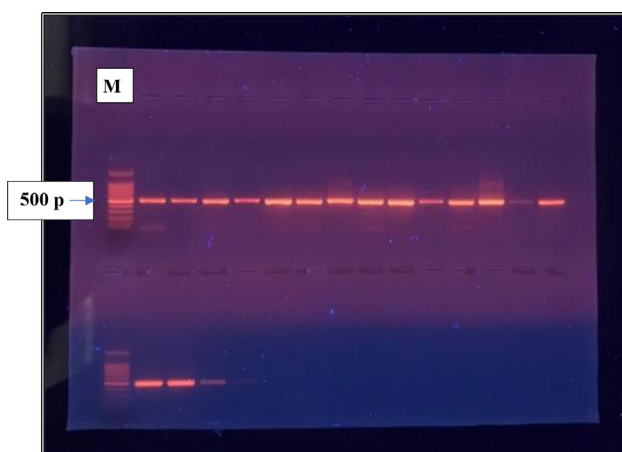


Fig. 7 PCR products of the 16S rRNA gene of some actinomycete isolates detected on 1% agarose gel electrophoresis and amplified using two primers, 27F and 519R, Lane M: 100 bp DNA ladder.

Molecular Identification

The isolates were identified by sequencing PCR products and the PCR amplification for some isolates by agarose gel electrophoresis is shown in Figure 7. The 16S rRNA sequences of the isolates were compared to the GenBank database at NCBI using the BLAST program. The results obtained from the direct sequencing of purified PCR products showed that about 91.6% of actinomycete isolates belonged to the genus

Streptomyces spp., and only one isolate, AL18, was supposed to be a species under the genus *Lentzea*. Table 1 showed the molecular identification of actinomycete isolates and the phylogenetic tree based on 16S rRNA sequence comparisons between the isolates using the neighbor-joining method was illustrated in Figure 8.

Screening for Enzyme Production

The results clarified that all isolates, except AL9 and AL10, exhibited good amylase production, while isolates AL9 and AL10 showed very weak production. However, all isolates were positive for protease, keratinase, gelatinase, chitinase, and lipase activity (Figure 9).

Screening for Antibacterial Activity

About 36.36% of actinobacterial cultures had antibacterial activity against at least one tested bacteria. As shown in Figure 10, approximately 14% of the total isolates could inhibit *S. aureus*. Also, 3 isolates (about 14%) had antibacterial activity against MRSA. Six isolates (27%) were active against *E. faecalis*. No inhibition zone or antibacterial activity was observed against all tested Gram-negative bacteria, *A. baumannii*, *K. oxytoca*, or *E. coli*. From these actinomycetes, isolate AL3 had the strongest inhibitory activity because it inhibited the growth of *S. aureus*, MRSA, and *E. faecalis* with inhibition zones of 11.0 ± 0.5 , 13.0 ± 0.5 , and 15.5 ± 0.5 mm, respectively (Table 2). Figure 11 illustrates the antibacterial activities of the selected actinomycetes against some Gram-positive bacteria.

Table 1. Molecular identification of arid area actinomycetes based on 16S rRNA gene sequence analysis

Isolate no.	Closest genus or species	Similarity (%)
AL1	<i>Streptomyces filamentosus</i>	100
AL2	<i>Streptomyces</i> sp.	98
AL3	<i>Streptomyces</i> sp.	94
AL4	<i>Streptomyces chilikensis</i>	100
AL5	<i>Streptomyces</i> sp.	98
AL6	<i>Streptomyces chilikensis</i>	100
AL7	<i>Streptomyces mutabilis</i>	95
AL8	<i>Streptomyces glaucescens</i>	97
AL9	<i>Streptomyces pseudogriseolus</i>	99
AL10	<i>Streptomyces sundarbansensis</i>	100
AL11	<i>Streptomyces thermodiastaticus</i>	98
AL12	<i>Streptomyces mutabilis</i>	100
AL13	<i>Streptomyces</i> sp.	99
AL14	<i>Streptomyces pseudogriseolus</i>	99
AL15	<i>Streptomyces werraensis</i>	99
AL16	<i>Streptomyces calvus</i>	100
AL17	<i>Streptomyces</i> sp.	99
AL18	<i>Lentzea albidocapillata</i>	99
AL19	<i>Streptomyces</i> sp.	98
AL20	<i>Streptomyces</i> sp.	97
AL21	<i>Streptomyces</i> sp.	97
AL22	<i>Streptomyces mutabilis</i>	100

Extraction of the Antibacterial Metabolites of Isolate AL3

Based on the significant antagonistic activity, isolate AL3 was chosen to extract antibacterial metabolites. This isolate showed the highest activity against the pathogenic bacterium *E. faecalis* using the agar well-diffusion method with an inhibition zone measuring 25 ± 0.5 mm (at a concentration of 100 μ l/well) as shown in Figure 12.

Discussion

Currently, an effective isolation strategy for extreme actinobacteria is lacking and improve selective isolation methods to target specific actinobacterial taxa of interest are still urgent needs. In this study, actinomycetes from extreme environments have attracted attention due to their special mechanisms of adapting to extreme conditions and because they can produce unusual compounds. It is postulated that tolerant isolates may have larger genetic and metabolic plasticity.⁵ The growing challenge of drug resistance emphasize the importance of exploring novel ecosystems to isolate actinobacterial strains, particularly *Streptomyces* species, for their production of bioactive secondary metabolites with significant pharmaceutical potential and novel enzymes that can become future harbingers of green biotechnology.²⁴

In the present investigation, 22 actinomycete colonies were obtained from an arid area about 35 km southeast of Jeddah, Bahrah Province, Saudi Arabia. Several factors were considered for the isolation of actinomycetes from the collected samples. Before diluting soils, the soils were air-dried at room temperature for about a week to decrease the population of Gram-negative bacteria because actinomycetes are

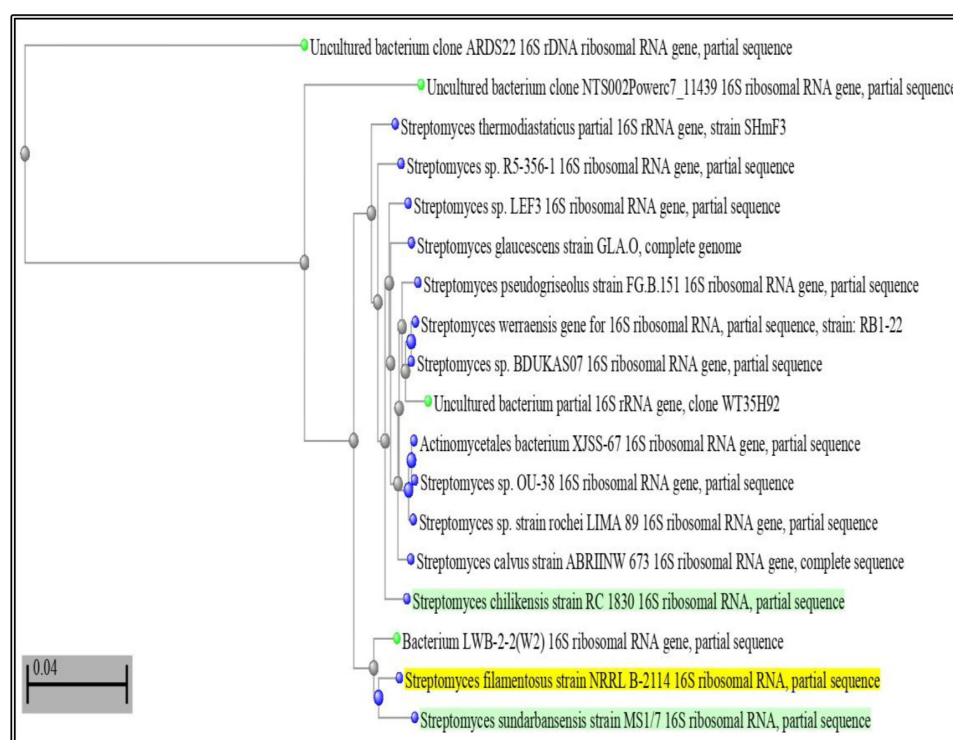


Fig. 8 Neighbor-joining tree based on 16S rRNA gene sequences showing the phylogenetic position of all actinomycetes obtained from the arid area, maximum sequence difference = 0.04.

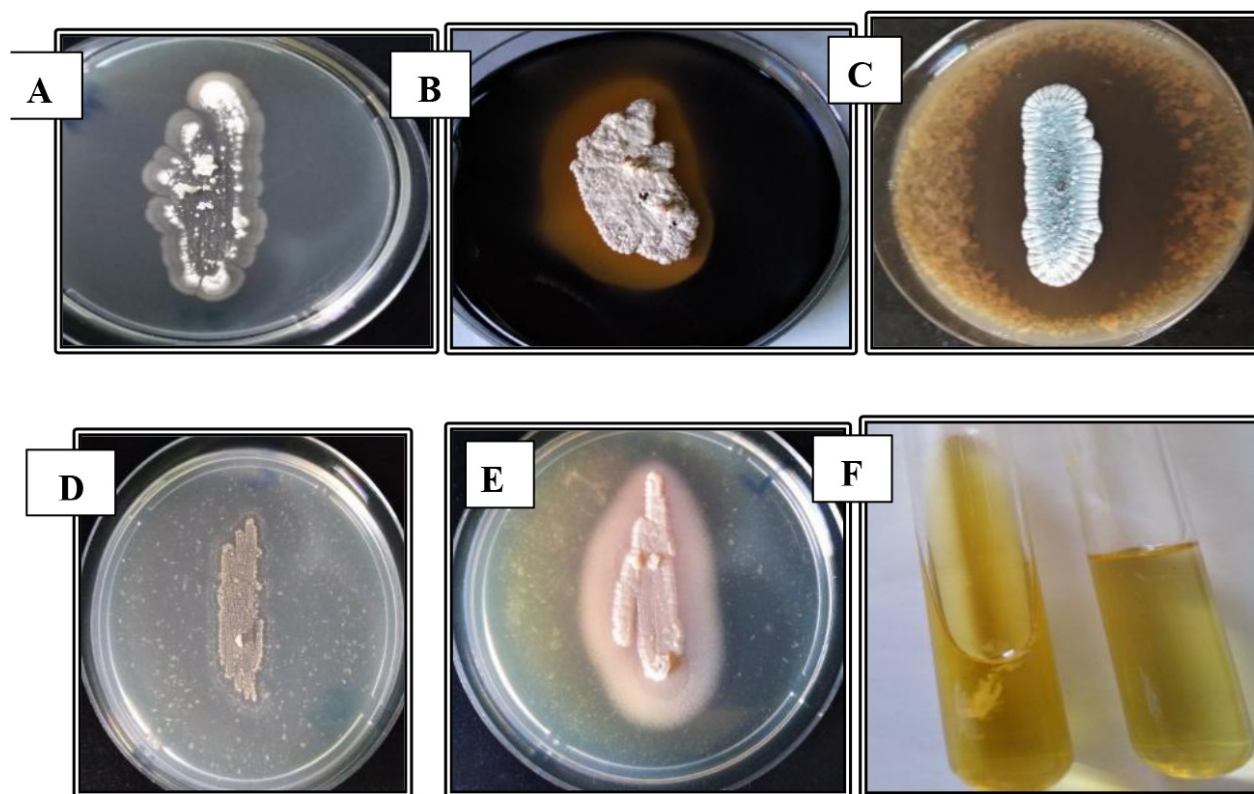


Fig. 9 The results of enzymes production by selected actinomycete isolates, (A): Keratinase degradation by isolate AL21, (B): Starch hydrolysis by isolate AL11, (C): protease production on skim milk by isolate AL2, (D): Lipolytic activity of isolate AL16 detected by a precipitate around the growth, (E): Chitinase activity of isolate AL10, and (F): Gelatin liquefaction by isolate AL1.

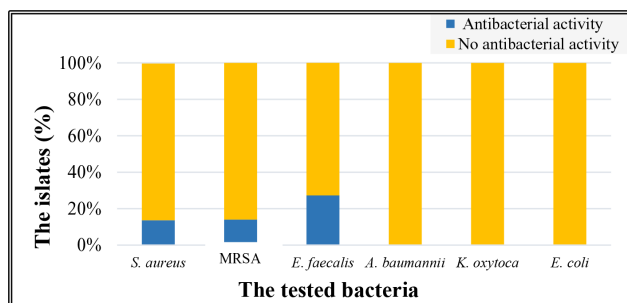


Fig. 10 Antibacterial activity of actinomycetes against Gram-positive and Gram-negative bacteria.

slow-growing relative to other soil bacteria. The samples were grown on a selective medium (starch nitrate agar), recommended for improving the isolation of actinomycetes.²⁵

Selective isolation procedures are often crucial for isolating new actinomycetes and eliminating unwanted microorganisms.²⁶ The preferential selection of actinomycetes using heat treatments might be related to the spore-forming capability of these groups, and phenol treatment of sample suspensions lowered the number of bacteria, and actinomycetes. Similarly, 10 soil samples collected from different sites in Egypt were treated by dry heat at 110°C for an hour and phenol treatment to isolate desirable rare actinomycetes.²⁶ They reported that some physicochemical pretreatments, such as thermal and phenol pretreatments can be used to isolate actinomycetes from different sources.

Table 2. The inhibitory activity of actinomycetes against three tested Gram-positive bacterial pathogens

Isolate no.	Zone of inhibition (mm)		
	<i>S. aureus</i> (ATCC 29213)	MRSA (ATCC 52923)	<i>E. faecalis</i> (ATCC 29212)
AL3 (control)	11.0 ± 0.5	13.0 ± 0.5	15.5 ± 0.5
AL5	6.0 ± 0.5*	-	6.0 ± 1.5*
AL9	-	12.5 ± 2.5	-
AL10	-	-	9.0 ± 0.5*
AL11	12.0 ± 0.5	-	-
AL14	-	10.0 ± 2.0*	11.5 ± 0.5
AL20	-	-	9.5 ± 0.5*
AL21	-	-	11.0 ± 0.5

The data were expressed as the mean value ± SD, -: No inhibition zone was detected under the study condition, *: Significant results at $P \leq 0.05$.

Research has shown that the use of selective isolation procedures reduces the population of actinomycetes, leading to the emergence of resistant isolates. In hyper-arid deserts, extreme desiccation is often accompanied by high temperatures, low nutrient concentrations, limited water activity, and intense radiation. Conversely, some arid ecosystems are characterized by low temperatures along with high salinity,

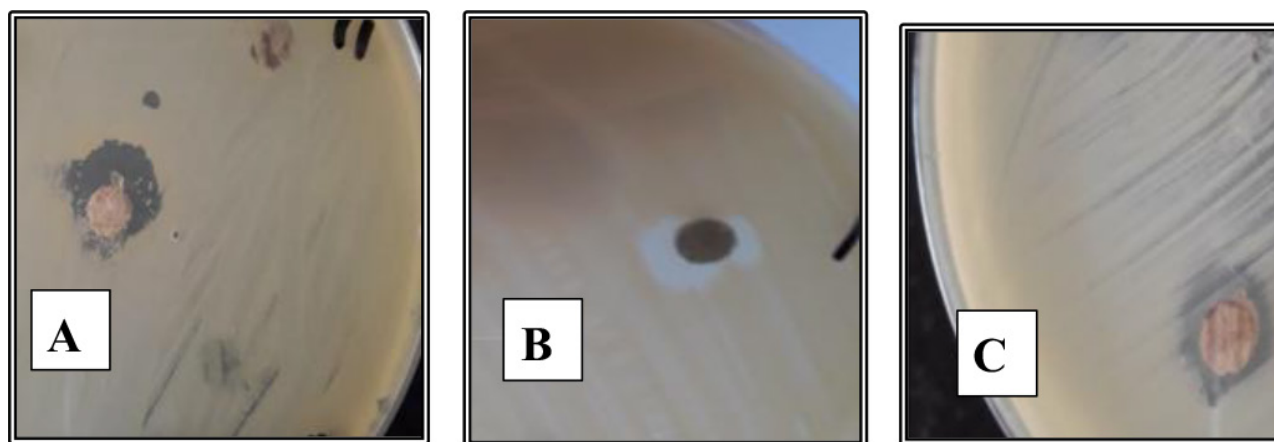


Fig. 11 The antagonistic activities of some actinomycete isolates, A: Isolate AL11 inhibited the growth of *S. aureus*, B: Isolate AL14 inhibited the growth of MRSA, and C: Isolate AL20 inhibited the growth of *E. faecalis*.



Fig. 12 An inhibition zone of isolate AL3 extract against *E. faecalis* grown on Mueller-Hinton agar and DMSO as a control.

pH levels, and concentrations of metals, nitrates, sulfates, or inorganic oxidant anions. There is substantial evidence that microorganisms in desert environments can be metabolically active rather than being dormant or nonfunctional.²⁷

In this study, arid region actinomycetes were identified using different characters in addition to 16S rRNA sequencing analysis. The obtained data revealed that the most frequently isolated genus was *Streptomyces* with various species; one isolate belonged to the genus *Lentzea*. This finding agreed with previous studies that recorded the presence of actinomycete colonies in different arid regions, such as desert soils. Members of the genus *Streptomyces* were reported from the hyper-arid Atacama Desert, such as *S. deserti*, *S. bullii* and *S. atacamensis*.^{7,8} More genera such as *Streptomyces*, *Amycolatopsis*, *Nocardia*, and *Actinomadura* were reported from arid habitats.⁵ Similarly, Kurapova et al.⁶ recorded that *Streptomyces*, *Micromonospora*, *Actinomadura*, and *Streptosporangium* were the most widespread in desert soils.

In the present investigation, arid area actinomycetes were tested for their capacity to produce some important enzymes.

Of the total isolates, approximately 90.90% of the isolates were amylase producer, and all isolates produced protease, keratinase, gelatinase, chitinase, and lipase, which are of biotechnological and industrial importance. Similarly,²⁸ reported that *Streptomyces* are being recognized as the best-known producers of critical enzymes like amylase, lipase, protease, keratinase, and chitinases.

During a drug discovery program, Schulz et al.²⁹ isolated a *Streptomyces* strain from Atacama Desert that produced novel aminobenzoquinones, active against bacteria and dermatophytes. Similarly, Yekkour et al.³⁰ isolated a desert *Streptomyces* strain, WAB9, that produced a novel molecule with antimicrobial activity against a selection of drug-resistant bacteria and yeasts. In another study, *Streptomyces leeuwenhoekii* produced two antibiotics, chaxamycins and chaxalactins³¹ while Chaxamycin A-D showed promising selective antibacterial activity against *S. aureus* and MRSA clinical isolates.³²

In this study, the isolated actinomycetes were more active against Gram-positive bacteria compared to Gram-negative bacteria, which may be due to differences in cell wall composition. Basilio et al.³³ recorded that Gram-negative strains were highly resistant. These findings agreed with the current study, which showed that 8 actinomycetes (36.36%) out of 22 isolates showed antibacterial activity against at least one of the tested Gram-positive bacteria. Like the present study, Dewedar et al.³⁴ isolated a thermophilic actinomycete, *Thermomonospora* sp. T-SA-125, from Saudi Arabian desert soil, is active against Gram-positive bacteria and is less active against Gram-negative bacteria. Asenjonamide C is a new polyketide antibiotic, isolated from *S. asenjonii*, and was active against *S. aureus*, *Bacillus subtilis*, *E. faecalis* and *E. coli*.³⁵ *Streptomyces* sp. DA3-7, isolated from the Saudi Arabian desert, provided a new pyridine alkaloid antibiotic active against *E. coli* and *Cryptococcus neoformans*.³⁶

In this study, the crude antibacterial compound from the most active isolate AL3 was extracted by ethyl acetate, and the dried extract in DMSO showed inhibition activity against *E. faecalis*. Recently, different solvents, such as ethyl acetate, chloroform, diethyl ether, and water were used for bioactive metabolite extractions and ethyl acetate was the best solvent due to its better extraction capability.³⁷

From all results, it may be highlighted that extreme habitats like arid areas in Saudi Arabia have proven to be a unique

habitat for isolation of actinomycetes, especially *Streptomyces* with antibacterial and enzyme activity. In agreement with this study, Sivalingam *et al.*³⁸ reported that potential *Streptomyces* species existed in all the extreme environments. Integrating new habitats with screening and improvement technologies can offer promising candidates for biotechnology and health-related applications.

Conclusions

Ultimately, the study on extremophile rare actinomycetes in Saudi Arabia is still at an early stage, and various bacteria that

are producers of useful bioactive compounds and enzymes remain to be discovered to be a magic solution for most pathogens that become resistant to antibiotics. Actinobacteria members, especially *Streptomyces* spp., have long been recognized as prolific sources of useful bioactive metabolites and continue to be a rich resource for new bioactive compounds. Therefore, current actinomycetes isolation programs are reoriented toward largely unexplored and extreme environments.

Conflict of Interest

None. ■

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