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Antimicrobial Activity of Ten Local Actinobacterial Strains against ESKAPE, *Bacillus subtilis* and *Pseudomonas baetica* Pathogens

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: The study included the isolation, purification, cultural characteristics, antimicrobial activities and molecular identification of local actinobacterial strains isolated from different locations north of Iraq.
Methodology: Oligotrophic medium supplemented with the antifungals cycloheximide (50 mg/l) and nystatin (30 mg/l) was used for preliminary isolation. ISP-3 medium was chosen as a potential medium for subsequent purification of actinobacterial strains. The cultural characteristics of all isolated actinobacterial strains were elucidated on International Streptomyces Project media (ISP2-ISP-7). 16S rRNA marker gene was used for molecular identification using 27F and 1492R universal primers.
Results: Ten isolates were biologically active against tested ESKAPE, *Bacillus subtilis* and pseudomonas paetica pathogens when cultured on different ISPs media under the OSMAC approach. Six representative isolates that exhibited antimicrobial activity against all or almost tested bacteria were sequenced using 16S rRNA gene. The sequences were compared with those of other actinobacterial strains that are found in Genebank database to find the best similarity and the close reference strains to our isolates. Five of the sequenced strains have been identified as Streptomyces species; MT5, MT8, MT12, MT23 and MT26 and one was identified as a rare actinobacterial strain Lentzea sp.; MT4. Nucleotide sequences have been provided and deposited in the National Center for Biotechnology Information NCBI under the accession numbers ON514131, ON514133, ON514134, ON514135, ON514136 and ON514130 respectively.

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Keywords: Actinobacteria; antimicrobial activities; ESKAPE pathogens; streptomyces spp.; 16S rRNA.

1. INTRODUCTION

The rising levels of antimicrobial resistance is one of the global threats of the current century. It is estimated that by the year of 2050, infections caused by resistant bacterial strains will lead to 300 million deaths prematurely [1]. The Centers for Disease Control and Prevention (CDC) reported that antibiotic-resistant infections account for two million illnesses and 23,000 deaths annually in the US, with over $20 billion as direct health-care costs and $35 billion in lost productivity [2]. Bacterial strains can be naturally resistant to a particular antibiotic or become resistant through the acquisition of resistance determinants [3]. The problem is further complicated by the lack of new and effective antibiotics under development against multi-drug resistant (MDR) pathogens [4,5].

Market demand for new drugs is extremely urgent and extensive due to common ailments and the fast spread of diseases, the development of new diseases with unknown causes, and the spread of antibiotic-resistant pathogens [6]. With the increasing advancement in science and technology, it is predicted that there would be a greater demand for new bioactive compounds synthesized by actinobacteria from various sources including soil and marine [7]. Genera of actinobacteria are well-known as rich sources in the discovery of important bioactive secondary metabolites including antibiotics, immunosuppressive drugs, anticancer drugs, and other biologically active compounds [8,9]. Genera such as *Streptomyces*, *Saccharopolyspora* and *Salinispora* were underestimated for years based on traditional isolation approaches.

It has been estimated that a total of 70,000 microbial secondary bioactive metabolites are known, about 40,000 have been originated from bacteria, of which approximately half (20,000) are isolated from actinobacteria [10]. Moreover, two-third of all antibiotics currently used in clinical purposes have been produced by actinobacteria [11].

After the discovery of the penicillin, the first antibiotic isolated from the Pnicillium notatum fungus, by Sir Alexander Fleming in 1928 [12], intensive efforts from the microbiologists around the world have been carried out to isolate and screen for new microbial strains particularly from actinobacteria or for new bioactive metabolites. A few years later, three antibiotics from the *Streptomyces* genus actinomycin from *Streptomyces antibioticus*, streptothricin from *Streptomyces lavendulae*, and streptomycin from *Streptomyces griseus* were discovered by Selman Waksman and his colleagues [13]. Chloramphenicol from *Streptomyces venezuelae* and tetracycline from *Streptomyces rimosus* were also discovered at that time [14].

In addition, researchers have isolated different genera of actinobacteria that produced valuable antibiotics and bioactive compounds such as rifamycins from *Amycolatopsis mediterranei*, erythromycin from *Saccharopolyspora erythraea*, teicoplanin from *Actinoplanes teichomyceticus*, vancomycin from *Amycolatopsis orientalis*, lupinacidins from *Micromonospora lupine*, neorustmicin from *M. carbonacea*, rifamycin from *M. rifamycinica*, gentamicin from *M. purpurea* and erythromycin from *Actinopolyspora* sp. [14,15]. Nonetheless, *Streptomyces* genus has been reported to be the tremendous source for bioactive natural products with clinical and pharmaceutical applications and has been reported to produce more than 75% of overall bioactive compounds produced by the other genera of actinobacteria [10].

Numerous studies have reported that vital and promising bioactive compounds are still isolated from many strains of actinobacteria and inhibited drug resistant pathogenic bacteria such as *Proteus mirabilis*, carbapenem *Acinetobacter baumannii*, vancomycin-resistant Enterococci, methicillin-resistant *Staphylococcus aureus*, *Shigella dysenteriae*, *Klebsiella* sp., *Escherichia coli*, and *Pseudomonas aeruginosa* [16,17].

However, the number of new classes of antibiotics that reached the final stage in the marketplace and clinical use are very few. Many scientific and economic concerns among the most causing delay in appearance and proving for new antibiotics. It was estimated that 10-15 years might take to prove an antibiotic for human use and it might cost of 1.3 billion USD from concept to the marketplace. [18]. Furthermore, 90% of the testing drug compounds proceed to clinical trials are failed in the first stage of the human testing [19].

2. MATERIALS AND METHODS

2.1 Biological Materials

For bioactive actinobacteria isolates, ten environmental samples originated from selected sites and mountains in Mosul, Dohuk, Sulaymaniyah and Erbil cities, Iraq, were collected in October, 2021. The samples were stored at room temperature until use. Before processing, the soils were air-dried at 100 °C for 60 minutes in a hot air oven and then cooled to room temperature. This helps in decreasing the population of gram negative bacteria and fungi. The soil samples were crushed, mixed thoroughly and large debris were removed to obtain fine soil particles, these samples were used for isolation of actinobacteria. For antimicrobial activity assay, the following ESKAPE bacterial reference strains of *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 43300, *Klebsiella pneumoniae* ATCC 700603, *Acinetobacter baumannii* ATCC 19606 and Pseudomonas aeruginosa ATCC 27853, and two more reference strains of *Bacillus subtilis* ATCC 23857 and *Pseudomonas baetica* a390 were tested.

2.2 Isolation of Actinobacterial Isolates

For each collected sample, the soil (1gm) was added to a plane tube (10 ml) containing 9 ml distill water. The tubes were vortexed for several minutes. Mixtures were allowed to settle at room temperature for 15 min. A series of dilutions of the suspension 10^{-2} and 10^{-3} in a total volume of 10 ml were prepared. An aliquot of 100 µl of each dilution was pipetted and spread evenly over the surface of oligotrophic medium (Wang et al., 2014) as a selective medium. The antifungals cycloheximide (50 mg/l) and nystatin (30 mg/l) were supplemented to inhibit the development of invasive fungi. The plates were incubated at 30°C in aerobic condition, and monitored over two to three weeks.

2.3 Purification of Actinobacteria

All screened samples are suspected to be inhabited with actinobacteria and the isolates showed actinobacteria like characteristic morphology on selective media were transferred onto fresh medium of ISP-3 medium [20] without adding antibiotic. Subculture was repeated on fresh ISP-3 medium, until pure isolates were obtained from each visible growth.

2.4 Cultural Characteristics of Actinobacterial Strains

Cultural characteristics of the pure isolates were determined by naked-eye examination according to the method described by Shirling and Gottlieb (1972) [21]. Cultural characteristics of the growth, aerial mass colour, reverse colour, diffusible pigment and melanin production were studied on ISP-2 to ISP-7 agar media (that contain trace salt solution) after growing at 30°C for 14 days. RAL K7 (edition 2012, Germany) classic code was used to describe all relevant colours.

2.5 Antibiotic Production Screening Assay by Actinobacteria

To discover the potential production of antibiotic compounds of the isolated actinobacteria utilising different media, all isolates were grown on ISP-2, ISP-3, ISP-4, ISP-5, ISP-6 and ISP-7 [20] for over two weeks at 30°C. The isolates were tested against references ESKAPE pathogens as well as *B. subtilis*, and *P. baetica*. Three to four colonies from each reference strain were transferred to 5 ml of normal saline, in a cell density equivalent to McFarland tube (0.5), and vortexed for a few seconds, then inoculate on nutrient agar medium by swabs. Then after, plugs of each actinobacterial cultures grown on different ISPs media plus a control plug containing only agar, were taken under sterile conditions using a cork borer, and placed over the nutrient agar plates containing the tested pathogen. The antibiotic production was evaluated by observing the inhibition zone between the target microorganisms and the plugs of cultures, after incubation at 37 °C for 24 hours.

2.6 Molecular Identification of Actinobacterial Strains

2.6.1 DNA extraction

The procedure of Presto™ Mini gDNA Bacteria Kit (Geneaid, Taiwan) was used for genomic DNA extraction. Briefly, actinobacterial isolates were grown on ISP-3 agar plates for seven days at 30 °C. The mycelia at the edge of the colonies were taken by loop and resuspended in Gram+ Buffer (200 µl/sample) to 1.5 ml Eppendrof tube and added lysozyme (0.8 mg/200 µl) then vortex to completely dissolve the lysozyme. Tubes were then incubated at 37°C for 30 minutes. During incubation, tubes were inverted every 10 minutes. Then, 20 µl of Proteinase K was added

and incubated at 60°C for at least 10 minutes. During incubation, tubes were inverted every 3 minutes. For lysis, DNA binding, washing and elution steps were followed as the procedure. The concentration and purity of extracted nucleic acids were determined using the NanoDrop UV-Vis spectrophotometer 2000c at 260/280 nm. The extracted DNA was stored at -20°C.

2.6.2 16S rRNA gene sequencing

Extracted genomic DNA samples were shipped to the UK (GENEWIZ, Azenta Life Sciences, Essex, UK) to be sequenced. Sequencing was carried out using an ABI 3730 XL automatic DNA sequencer. For 16S rRNA gene, GENEWIZ PCR primers service was used for this purpose. The 16S rRNA gene was amplified by PCR using the universal primers: 27F (5´- AGA GTT TGA TCC TGG CTC AG - 3´) and 1492R (5´- TAC GGC TAC CTT GTT ACG ACT T - 3´) [22].

2.6.3 BLASTn to search for gene sequences

The Nucleotide Basic Local Alignment Search Tool (BLASTn) program [23] was used to search for homology to the input template sequence against entire sequences which are available on the sequence databases. The default was 100 hits and Nucleotide BLAST (nr/nt) web database was chosen, and the program selection was set to highly similar sequences (megablast). Most closely related homologues sequences which had ≥96% sequence identity and had query coverage ≥99% with an E value 0.0 and met the criteria of aligning were identified.

3. RESULTS AND DISCUSSION

After a two to a three week of incubation on oligotrophic medium, actinobacterial like colonies were selected and isolated. Since our focus on isolating actinobacterial strains that might have antimicrobial activities, many unwanted microorganisms not like actinobacterial phenotypic characteristics were excluded; thus, only a very few number of isolates are finally chosen. Criteria of slow growing, aerobic, chalky, heaped, folded with aerial and substrate mycelia of different colours were considered for the isolation. For purification, ISP-3 medium was used. This medium was recommended as an important medium that support abundant growth of most actinobacterial strains [24]. Moreover, Oat (*Avena sativa L*.) is available, very cheap, easy to prepare and a rich source of valuable nutritional components such as carbohydrates, amino acids, lipids, phenolic compounds, vitamins and minerals [25].

To elucidate the cultural characteristics on different media, the isolated actinobacterial strains were grown on ISP-2, ISP-3, ISP-4, ISP-5, ISP-6 and ISP-7. It has shown that each isolated strain exhibited different growth characteristics on different ISPs media (Table 1). Growth, aerial mass colour, reverse colour, diffusible pigment and melanin production are among the most morphological features that are recommended for the description of actinobacterial isolates [21]. However, the abundant of growth, aerial mass colour, reverse side colour and soluble pigment production of any of the isolates have been revealed to be influenced by the medium components, growth conditions, and age of culture. Each ISP medium has different carbohydrate and/or amino acids sources and this might have an effect on metabolic pathways of pigment production. All the actinobactrial strains were tested for antibiotic production using agar plug technique. This technique is widely used for screening large numbers of bacterial isolates for their antibacterial activities [26]. If the bioactive compound is produced by a certain isolate of interest, then it will be diffused into the agar medium and consequently will prevent the tested bacterial pathogen from growing and this can be detected by observing an inhibition zone around the agar plug of the bioactive isolate.

As illustrated in Table (2) ten actinobacterial isolates were found to be biologically active against the tested bacterial species including some of Gram-negative and Gram-positive ESKAPE pathogens. It can be seen that isolates MT5, MT8, MT12 and MT29 produce bioactive compounds which have strong activities against all the tested pathogens. MT4, MT23, MT26, MT27 and MT36 isolates were found to inhibit the growth of all tested pathogens except for Gram-negative bacterium *P. baetica* which was not inhibited. MT10 isolate was not able to inhibit the growth of Gram-negative bacterium *A. baumannii* while it was able to inhibit the growth of other pathogens.

Natural bioactive compounds derived from actinobacterial strains are known to have more antimicrobial effect on Gram-positive bacteria than on Gram-negative bacteria [27]. This effect might be because that Gram-negative bacteria have different cell membrane structure which play an important role in these type of bacteria by preventing the bioactive compounds and antibiotics from penetrating the outer membrane and reach their target mode of action [28,29].

Table 1. Cultural characteristics of actinobacterial strains on ISPs media

Isolate No.	ISP-2	ISP-3	ISP-4	ISP-5	ISP-6/ISP-6
MT4	G: Poor A: Single White (RAL9003) R: None D: None	G: Abundant A: Single yellow (RAL1003) R: Sand Yellow (RAL1002) D: Sand Yellow (RAL1002)	G: Abundant A: Pearl Mouse Grey (RAL 7048) R: None D: None	G: Moderate A: Light Ivory (RAL 1015) R: None D: None	M: None produced
MT5	G: Moderate A: Wine Red (RAL3005) R: None D: None	G: abundant A: Ruby Red (RAL3003) R: Sand Yellow (RAL1002) D: Sand Yellow (RAL1002)	G: Abundant A: Purple red (RAL3004) R: None D: None	G: Moderate A: Oxide Red (RAL 3009) R: None D: None	M: None produced
MT8	G: Moderate A: Pearl Copper(RAL8029) R: Beige red (RAL3012) D: Beige Red (RAL3012)	G: Abundant A: Beige Red (RAL3012) R: Beige Red (RAL3012) D: Beige Red (RAL3012)	G: Abundant A: Traffic Grey (RAL7042) R: Cream (9001) D: None	G: Abundant A: Pastel Orange(RAL2003) R: None D: None	M: Produced
MT10	G: Abundant A: Traffic White (RAL9016) R: Beige Brown (RAL8023) D: None	G: Abundant A: Traffic White (RAL9016) R: Grey Beige (RAL1019) D: None	G: Abundant A: Traffic White (RAL9016) R: None D: None	G: Moderate A: Traffic White (RAL9016) R: None D: None	M: Produced
MT12	G: Moderate A: Pure white (RAL 9010) R: None D: None	G: Abundant A: Traffic White (RAL 9016) R: (RAL1001) D: None	G: Abundant A: Traffic White (RAL 9016) R: None D: None	G: Moderate A: Pure White (RAL 9010) R: None D: None	M: Produced
MT23	G: Moderate A: Pure White (RAL 9010)	G: Abundant A: Pure white (RAL 9010)	G: Moderate A: Pure White (RAL	G: Moderate A: Light Ivory (RAL 1015)	M: Produced

Isolate No.	ISP-2	ISP-3	ISP-4	ISP-5	ISP-6/ISP-6
	R: None D: None	R: Brown Beige (RAL 1011) D: Brown Beige (RAL 1011)	9010) R: None D: None	R: None D: None	
MT26	G: Moderate A: Salmon Orange (RAL 2012) R: Orange Brown (RAL 8023) D: Orange Brown (RAL 8023)	G: Abundant A: Signal Grey (RAL 7004) R: Ochre Brown (RAL 8001) D: Ochre Brown (RAL 8001)	G: Moderate A: Signal Grey (RAL 7004) R: None D: Grey Beige (RAL 1019)	G: Moderate A: Salmon Orange (RAL 2012) R: None D: None	M: Produced
MT27	G: Moderate A: Pure White (RAL 9010) R: Zinc Yellow (RAL 1018) D: Zinc Yellow (RAL 1018)	G: Abundant A: Pure White (RAL 9010) R: Beige Red (RAL 3012) D: Beige Red (RAL 3012)	G: Moderate A: Pure White (RAL 9010) R: None D: None	G: Moderate A: Heather Violet (RAL4003) R: Heather Violet (RAL4003) D: Heather Violet (RAL4003)	M: None produced
MT29	G: Abundant A: Beige Brown (RAL 8024) R: Orange Brown (RAL 8023) D: Orange Brown (RAL 8023	G: Abundant A: Salmon Orange (RAL 2012) R: Beige Red (RAL 3012) D: None	G: Abundant A: Beige Red (RAL 3012) R: Beige Red (RAL 3012) D: Beige Red (RAL 3012)	G: Moderate A: Beige Red (RAL 3012) R: None D: None	M: Produced
MT36	G: Abundant A: Broom Yellow (RAL 1032) R: None D: None	G: Abundant A: RAL 7093 R: Dusty Grey (RAL7037) D: Dusty Grey (RAL7037)	G: Abundant A: Golden Yellow (RAL 1004) R: None D: None	G: Moderate A: Grey Beige (RAL 1019) R: None D: None	M: None produced

Table 2. Bioassay using agar plug technique of ten actinomycete isolates grown on ISP media that exhibited antimicrobial activity against tested bacteria

E. coli ATCC 25922		*S. aureus* ATCC 43300		*K. pneumoniae* ATCC 700603		*A. baumannii* ATCC 19606		*P. aeruginosa* ATCC 27853		*B. subtilis* ATCC 23857		*P. baetica* strain a390	
Isolate No.	**ISP-Medium No.**	**Isolate No.**	**ISP-Medium No.**	**Isolate No.**	**ISP-Medium No.**	**Isolate No.**	**ISP-Medium No.**	**Isolate No.**	**ISP-Medium No.**	**Isolate No.**	**ISP-Medium No.**	**Isolate No.**	**ISP-Medium No.**
MT4	2, 3	MT4	2,3,4,5,7	MT4	2, 3	MT4	3, 4, 5	MT4	2, 3, 4, 5	MT4	3, 4, 5		
MT5	2, 3, 4, 5	MT5	2,3,4,5,7	MT5	3, 4, 5, 7	MT5	3	MT5	2, 7	MT5	2, 3, 4, 5,7	MT5	2,3,4,5,7
MT8	2, 3, 4 ,7	MT8	2,3,4,7	MT8	2, 3, 4, 7	MT8	3, 4, 7	MT8	2, 3, 4, 7	MT8	2, 3, 4, 7	MT8	2, 3, 4, 7
MT10	2, 3, 4, 7	MT10	2,3,4,7	MT10	2,3,4,7			MT10	3, 4	MT10	2, 4	MT10	2, 3, 4, 7
MT12	2,3,4,5,7	MT12	2,3,4,5,6,7	MT12	2,3,4,5,7	MT12	2,3,4,5,7	MT12	2,3,4,5,7	MT12	2, 3, 4, 5,7	MT12	2,3,4,5
MT23	3, 4, 5, 7	MT23	2,3,4,5,7	MT23	3, 4, 7	MT23	3, 4, 7	MT23	3, 4, 7	MT23	3, 4, 5		
MT26	3, 4, 7	MT26	2,3,4,7	MT26	3, 4, 7	MT26	3, 4, 7	MT26	3, 4, 7	MT26	3, 4, 7		
MT27	4, 5, 7	MT27	3,4,5,6,7	MT27	3, 4, 5, 7	MT27	3, 4,5, 7	MT27	3, 4, 5, 7	MT27	3, 4, 5, 6,7		
MT29	2,3,4,5,7	MT29	2,3,4,5,6,7	MT29	2,3,4,5,7	MT29	2,3,4,5,7	MT29	2,3,4,5,7	MT29	2, 3, 4, 5,7	MT29	2,3,4,5
MT36	2, 3, 5, 7	MT36	2,3,4,5,7	MT36	2,3,4,5,7	MT36	2, 4, 7	MT36	2, 3, 5, 7	MT36	2,3,4,5,6,7		

Table 3. Reference strains with their accession numbers that represented the highest homology to each of the six bioactive actinobacteria isolates as determined by, query coverage, E-value, percentage identity, Query / Subject Length and accession number

Isolate No.	Most Closely Strain (accession number)	Query Coverage	E-value	Percentage Identity	Query / Subject Length	Accession Number
MT4	*Lentzea albidocapillata* (EU593721.1)	99%	0.0	97.89%	1394 / 1424	ON514130
MT5	*Streptomyces* sp. strain IHBB 9327 (KU921579.1)	99%	0.0	96.23%	1382 / 1436	ON514131
MT8	*Streptomyces* sp. strain EIIIA (MN964268.1)	99%	0.0	99.19%	1351 / 1362	ON514133
MT12	*Streptomyces* pseudovenezuelae (MT083995.1)	99%	0.0	99.36%	1397/ 1426	ON514134
MT23	*Streptomyces aurantiacus* strain QMA67 (MT525239.1)	98%	0.0	97.96%	1032 / 1436	ON514135
MT26	*Streptomyces vinaceus* strain movR-5 (KY753358.1)	99%	0.0	97.31%	1377 / 1415	ON514136

It can be seen that the actinobacterial isolates displayed a different range of antimicrobial activity based on the cultural media they were grown on. As shown in Table (2), a large number of actinobacterial isolates presented bioactivity against tested pathogens when cultured on ISP3 and ISP4 media. The results also showed that actinobacterial isolates grown on ISP2, ISP3, ISP4, ISP5 and ISP7 were active on both Gram-negative and Gram-positive tested pathogens. Interestingly, none of the actinobacterial isolates showed bioactivity against any of Gram-negative pathogen when cultured on ISP6, while isolates MT12, MT27, MT29, MT36 were active against Gram-positive pathogens *S. aureus*, and *B. subtilis* respectively. From these results, in can be concluded that the actinobacterial isolates activated to produce bioactive compounds due to nutrients variation in culture media compositions. In this regard, each ISP medium contains different carbon and nitrogen sources. The variation in carbon and nitrogen supplements have been implemented under the One Strain Many Compounds (OSMAC) approach. In this approach small changes in medium composition of an interest bacterial or fungal isolate can result in big effects in the production several natural secondary metabolites [30]. In a study conducted by Rateb et al. [31] on Streptomyces sp. strain C34, three novel chemical metabolites with significant action against some pathogenic bacteria were discovered utilizing the OSMAC approach within the same *Streptomyces* strain by varying the carbon and nitrogen sources. In another recent study, diverse bioactive compounds that have a potent antimicrobial activity against MDR bacteria were identified from *Streptomyces* sp. strain YINM00001 which was isolated from a Chinese medicinal herbal plant by application of the OSMAC approach [32]. Nevertheless, to avoid the complexity of the medium composition for the secondary metabolites produced by an interesting actinobacterial isolate, biosynthesis of bioactive compound has to be screened on a poor medium such as a minimum medium supplemented with various nitrogen and/or carbon precursors independently.

Six representative isolates (Table 3) that exhibited antimicrobial activity against all or almost tested bacteria were compared with those of other actinobacterial strains that are found in Genebank database based on 16S rRNA gene similarities. The results showed that none of the six sequenced isolate showed 100% degree of identity-similarity with any reference strain. However, the representative isolates MT4, MT5, MT8, MT12, MT23 and MT26 showed 97.89%, 96.23%, 99.19%, 99.36%, 97.96% and 97.3% identity respectively; therefore, this suggest that these isolates may be new strains of actinobacteria. However, for suspected novel strains showing 16S rRNA gene identity of 98.5% or higher, it is highly recommended to perform conventional DNA-DNA Hybridization for testing the relatedness [33]. Moreover, new bioinformatics methods such as *in silico* DNA-DNA Hybridization and Average Nucleotide Identity (ANI) have been widely accepted and used by microbiologists' community for assigning new bacterial strains based on the degree of similarity between the genomes of two very closely strains obtained from whole genome sequencing data [34,35].

4. CONCLUSION

Actinobacteria are well-known to be animportant source of useful secondary metabolites and new antibiotics. In this study, ten new actinobacteria species belong to two genera of Streptomyces and Lentzea from Iraqi soils were isolated and identified. The 16S rRNA gene of six representative strains were sequenced and submitted to the NCBI Genbank database. The study showed that oligotrophic medium was excellent for preliminary isolation of actinobacterial isolates, whereas ISP-3 medium gave excellent results in purification as it supported the growth of all purified actinobacterial strains. The isolates displayed different patterns of morphology and physiology when grown on on a full set of ISPs (ISP-2-ISP-7) media supplemented with different chemical sources. Promising local actinobacterial isolates were activated to produce bioactive compounds under the OSMAC approach against Gram-negative and Gram-positive ESKAPE pathogens, *B. subtilis* and *P. baetica* utilizing the variation in carbon and nitrogen supplements of ISPs media. Iraqi environments are rich sources for promising novel actinobacterial strains that have potential to produce new medically bioactive compounds. The universal 16S rRNA gene primers 27F and 1492R were successfully amplified in detecting the actinobacterial genera including *Streptomyces* and the rare actinobacteria as in case of *Lentzea*.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. O'Neill J. Tackling drug-resistant infections globally: final report and recommendations; 2016.
Accessed 5 August 2022.
Available:http://amr-review.org/sites/default/files/160525_Final%20paper_with%20cover.pdf
2. Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States, 2013. Centers for Disease Control and Prevention, US Department of Health and Human Services, Atlanta, GA; 2013.
Accessed 12 September 2022.
Available: https://www. cdc. gov/drugresistance/threat-report-2013/pdf/arthreats-2013-508. pdf
3. Monserrat-Martinez A, Gambin, Y, Sierecki, E. Thinking outside the bug: molecular targets and strategies to overcome antibiotic resistance. International Journal of Molecular Sciences. 2019;20(6):12-20.
4. Cillóniz C, Dominedò C, Torres A. Multidrug resistant gram-negative bacteria in community-acquired pneumonia. Annual Update in Intensive Care and Emergency Medicine 2019. 2019;459-475.
5. Wang Z, Koirala B, Hernandez Y, Zimmerman M, Park S, Perlin DS, Brady SF. A naturally inspired antibiotic to target multidrug-resistant pathogens. Nature. 2022;601(7894):606-611.
6. Cook MA, Wright GD. The past, present, and future of antibiotics. Science Translational Medicine. 2022;14(657): eabo7793.
7. Azman AS, Othman I, Velu SS, Chan KG, Lee LH. Mangrove rare actinobacteria: Taxonomy, natural compound, and discovery of bioactivity. Frontiers in microbiology. 2015;6:85-96.
8. Selim MS, Abdelhamid SA, Mohamed SS. Secondary metabolites and biodiversity of actinomycetes. Journal of Genetic Engineering and Biotechnology. 2021; 19(1):1-13.
9. Tan LT, Chan KG, Pusparajah P, Yin WF, Khan TM, Lee LH, Goh BH. Mangrove derived Streptomyces sp. MUM265 as a potential source of antioxidant and anticolon-cancer agents. BMC microbiology. 2019;19(1):1-16.
10. Bérdy J. Thoughts and facts about antibiotics: Where we are now and where we are heading. The Journal of antibiotics. 2012;65(8):385-395.
11. van Bergeijk DA, Terlouw BR, Medema MH, van Wezel GP. Ecology and genomics of Actinobacteria: new concepts for natural product discovery. Nature Reviews Microbiology. 2020;18(10):546-558.
12. Aminov RI. A brief history of the antibiotic era: lessons learned and challenges for the future. Frontiers in microbiology. 2010; 1:34-39.
13. Waksman SA, Woodruff HB. Bacteriostatic and bactericidal substances produced by a soil Actinomyces. Proceedings of the society for Experimental Biology and Medicine. 1940;45(2):609-614.
14. Takahashi Y, Nakashima T. Actinomycetes, an inexhaustible source of naturally occurring antibiotics. Antibiotics. 2018;7(2):45-52.
15. Amin DH, Abdallah NA, Abolmaaty A, Tolba S, Wellington EM. Microbiological and molecular insights on rare Actinobacteria harboring bioactive prospective. Bulletin of the National Research Centre. 2020;44(1):1-12.
16. Terra L, Dyson PJ, Hitchings MD, Thomas L, Abdelhameed A, Banat IM, Quinn GA. A novel alkaliphilic Streptomyces inhibits ESKAPE pathogens. Frontiers in microbiology. 2018;24:58-64.
17. Quinn GA, Banat AM, Abdelhameed AM, Banat IM. Streptomyces from traditional medicine: Sources of new innovations in antibiotic discovery. Journal of Medical Microbiology. 2020;69(8):1040-1049.
18. Van Norman GA. Drugs, devices, and the FDA: part 1: An overview of approval processes for drugs. JACC: Basic to Translational Science. 2016;1(3): 170-179.
19. DiMasi JA, Grabowski HG, Hansen RW. Innovation in the pharmaceutical industry: New estimates of R&D costs. Journal of health economics. 2016;47:20-33.
20. Shirling EB, Gottlieb D. Methods for characterization of Streptomyces species.

International Journal of Systematic Bacteriology. 1966;16:313-340.
21. Shirling EB, Gottlieb D. Cooperative description of type strains of streptomyces. International Journal of Systematic Bacteriology. 1972;22:265-394.
22. Chun J, Goodfellow M. A phylogenetic analysis of the genus Nocardia with 16S rRNA gene sequences. International Journal of Systematic and Evolutionary Microbiology. 1995;45(2):240-245.
23. Altschul P, Gish W, Miller W, Myers E, Lipman D. Basic local alignment search tool. J. Mol. Biol. 1990;215:403-410.
24. Rajeswari P, Jose PA, Amiya R, Jebakumar SR. Characterization of saltern based Streptomyces sp. and statistical media optimization for its improved antibacterial activity. Frontiers in Microbiology. 2015; 5:75-87.
25. Paudel D, Dhungana B, Caffe M, Krishnan P. A review of health-beneficial properties of oats. Foods. 2021;10(11):25-35.
26. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. Journal of pharmaceutical analysis. 2016;6(2):71-79.
27. Stan D, Enciu AM, Mateescu AL, Ion AC, Brezeanu AC, Stan D, Tanase C. Natural compounds with antimicrobial and antiviral effect and nanocarriers used for their transportation. Frontiers in Pharmacology. 2021;24:82-89.
28. May KL, Grabowicz M. The bacterial outer membrane is an evolving antibiotic barrier. Proceedings of the National Academy of Sciences. 2018;115(36):8852-8854.
29. Zhang YB, Liu XY, Jiang PP, Li WD, Wang YF. Mechanism and antibacterial activity of cinnamaldehyde against Escherichia coli and Staphylococcus aureus. Mod. Food Sci. Technol. 2015;31:31-35.
30. Romano S, Jackson SA, Patry S, Dobson AD. Extending the “one strain many compounds”(OSMAC) principle to marine microorganisms. Marine drugs. 2018; 16(7):244-252.
31. Rateb ME, Houssen WE, Harrison WT, Deng H, Okoro CK, Asenjo JA, Jaspars M. Diverse metabolic profiles of a streptomyces strain isolated from a hyper-arid environment. Journal of natural products. 2011;74(9): 1965-1971.
32. Liu T, Ren Z, Chunyu WX, Li GD, Chen X, Zhang ZT, Yin M. Exploration of diverse secondary metabolites from streptomyces sp. YINM00001, Using Genome Mining and One Strain Many Compounds Approach. Frontiers in microbiology. 2022; 13:831174-831174.
33. Stackebrandt E, Rainey FA, Ward-Rainey N. Proposal for a new hierarchic classification system, Actinobacteria classis nov. International Journal of Systematic and Evolutionary Microbiology. 1997;47(2):479-491.
34. Varghese NJ, Mukherjee S, Ivanova N, Konstantinidis KT, Mavrommatis K, Kyrpides NC, Pati, A. Microbial species delineation using whole genome sequences. Nucleic acids research. 2015; 43(14):6761-6771.
35. Tortoli E. The new bacterial species description: The genus Mycobacterium as an example. Revista Pan-Amazônica de Saúde. 2017;8(4):2-12.

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