

Metabolites Profiling and Antimicrobial Activity of Actinobacteria Associated with Plants Collected from Thumama in Riyadh Region Saudi Arabia

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Abstract

Original Research Article

Plants are emerging as a vital source of microbial diversity and, therefore, of bioactive secondary metabolites. This study aimed to metabolomes profiling and investigate the antimicrobial activity of actinobacteria associated with plants collected from thumama desert in Riyadh region, Saudi Arabia. Assessment of the antimicrobial potential has been done using agar well diffusion method and the metabolites profiling has been carried out using HPLC and GC-MS. Several actinobacterial extracts showed antimicrobial activity against multiple tested microbes, NA118 and N182 exhibited antimicrobial activity against the three tested microbes *Klebsiella pneumoniae*, *Bacillus subtilis* and *Candida albicans*. Actinobacterial strains showed differential levels of amino acids, Isolate NA118 showed the highest increases in valine, proline, ornithine, leucine. Highest levels of hexadecatrienoic (C16:3) and hexadecanoic (C16:0) were recorded for isolate NA8. Isolate NA182 was rich in several phenolics and flavonoids. High contents of beta-tocopherol and delta-tocopherol and gama-tocopherol were recorded for isolate NA118 and NA18. Finally, The pharmacological characteristics of the bacterial extracts were assessed. Antibacterial testing revealed that 10 of 12 bacterial extracts were having antimicrobial activities against at least one of the microorganisms examined. Therefore, Further studies needs to be performed in which we can isolate and identify various actinobacterial species using different media beside it could have interesting biologically active metabolites.

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INTRODUCTION

Plants are emerging as a vital source of microbial diversity, with recent studies revealing that plant-associated microbial communities are diverse and complex (Bulgarelli *et al.*, 2013). These microbial communities include bacteria, fungi, and viruses that interact with the plant in various ways, such as promoting growth, enhancing nutrient uptake, and providing protection against pathogens. Plant-associated microbes are also known to produce a wide range of secondary metabolites with potential medicinal and industrial applications. As such, understanding the diversity and function of plant-associated microbial communities is becoming increasingly important for both basic and applied research. Advances in high-throughput sequencing technologies have enabled researchers to explore the plant microbiome in greater

detail, revealing a vast and dynamic ecosystem that is closely intertwined with the plant host.

Actinobacteria are an important component of the plant microbiome and play a vital role in plant growth and health (Barka *et al.*, 2016). As mentioned in the previous paragraph, plant-associated microbes are diverse and complex, including bacteria, fungi, and viruses. Actinobacteria are one of the major bacterial phyla found in the rhizosphere and phyllosphere of plants, and they are known to interact with the plant host in various ways, including promoting growth and providing protection against pathogens. Actinobacteria are also known to produce a wide range of secondary metabolites with potential applications in medicine, agriculture, and industry. Some of these secondary metabolites have been shown to have plant growth-promoting properties. Therefore, understanding

the diversity and function of actinobacteria in the plant microbiome is becoming increasingly important for both basic and applied research.

Actinobacteria are a diverse group of Gram-positive bacteria that have a filamentous or mycelial growth form. They are widespread and can be found in various environments such as soil, water, and plants (Barka *et al.*, 2016). Actinobacteria are well known for their ability to produce a wide range of bioactive compounds, including antibiotics, anticancer agents, immunosuppressants, and enzymes (Bérdy, 2012). These bioactive compounds have significant biotechnological potential and have been used in various applications, such as medicine, agriculture, and food industry.

The biotechnological importance of actinobacteria lies in their ability to produce a diverse range of bioactive compounds with therapeutic properties. Many clinically important antibiotics, such as streptomycin, erythromycin, and tetracycline, are produced by actinobacteria (Bérdy, 2012; Barka *et al.*, 2016). Actinobacteria are also a rich source of anticancer agents, such as doxorubicin and mitomycin C, and immunosuppressants, such as rapamycin. In addition, actinobacterial enzymes, such as amylases and cellulases, have been widely used in various industrial applications, such as food processing and biofuel production (Bérdy, 2012). The biotechnological potential of actinobacteria highlights the importance of understanding their biology and ecology, as well as developing efficient methods for their cultivation and exploitation. This study aimed to assess the antimicrobial activity and metabolite profiling of actinobacteria associated with plants collected from thumama desert in Riyadh region, Saudi Arabia.

MATERIALS AND METHODS

1. Study area and Sample collection:

Plants were collected from several locations in Thumama desert in Riyadh, Saudi Arabia. Plant materials were obtained from these plants. All samples were collected in a sterile plastic bag and then transported to our King Saud University laboratory for bacteria isolation.

2. Isolation and purification of Actinobacteria bacteria:

Plant parts were surface sterilized to remove all the surface-living organisms. Started with carefully washed with tap water to remove attached clay; followed this, the plant parts were sequentially immersed in 5% aqueous solution of sodium chloride, tissues were then washed with autoclaved distilled water to wash away the residues and epiphytic organisms.

Following that, the explants were washed with distilled water. Finally, 1 gm of sterilised plant tissues were combined with 2 mL of autoclaved distilled water

and ground in a mortar. Serial dilutions of the aqueous sterilised plant solution samples were generated by mixing 1 ml of the sample with 9 ml of sterilised distilled water (10^{-1}), stirring, and repeating the process until the concentration reached 10^{-5} . Each dilution was spread by smearing 100 microliters of the sample dilution onto the surface of isolation plates containing ISP2 and Modified Hagem media supplemented with nystatin (25 g mL^{-1}) to suppress fungal growth. The soil samples were also serially diluted by aseptically mixing 10 g of dirt in 90 mL of sterile distilled water (10^{-1}), mixing by shaking, and making tenfold dilutions up to 10^{-6} . The agar plates were spread with 0.5 mL aliquots of each soil from dilutions 10^{-4} , 10^{-5} , and 10^{-6} . Each dilution was duplicated twice on two plates. After that, the plates were kept at 35°C for 24 hours.

On a plate, the average colony count of bacteria produced was calculated. The results were represented as colony forming units per gramme of soil dry weight (CFU/g). Colonies were identified by their cultural and physical characteristics, and bacterial isolates were purified many times in the same isolation medium using the streaking method. These isolates' stock cultures were kept at 80°C in cryotubes with 1.5 mL 20 percent (w/v) sterile glycerol solution (Wellington and Williams, 1978).

3. Identification of the isolated endophytic bacteria:

Identification of strains morphologically was done by Electron microscope using Coverslip culture technique on both ISP2, as well as Hagem media agar (Yaminisudha *et al.*, 2015). 16S rRNA gene sequences were also used to identify the bacteria. Endophytic bacteria genomic DNA was isolated (Govindarajan *et al.*, 2007) and 16S rDNA was amplified in PCR using the genomic DNA as template and bacterial universal primers 27 F (5'-GAGTTTGAT CACTGGCTCAG-3') and 1492 R (5'-TACGGC TACCTTGTTACGACTT-3') (Byers *et al.*, 1998). After matching sequences with CLUSTAL X, (Kimura, 1980) by MEGA 5.0 programme (Tamura *et al.*, 2011), (Thompson *et al.*, 1997). With 1,000 replications, a bootstrap analysis was performed (Felsenstein, 1985). The sequences were compared to sequences of the 16S rRNA gene from cultured species available on the EzTaxon-e server1 (Yoon *et al.*, 2017). After matching sequences with CLUSTAL X, a phylogenetic tree was generated using the neighbor-joining (NJ) technique (Saitou and Nei, 1987) and Kimura's two parameter model (Kimura, 1980) by MEGA X software (Tamura *et al.*, 2011). (Thompson *et al.*, 1997). With 1,000 replications, a bootstrap analysis was performed (Felsenstein, 1985).

4. Screening of antimicrobial activities:

In nutritional broth and potato dextrose broth, bacterial and fungal cultures were produced, respectively. Gram-negative and positive bacteria such as *Klebsiella pneumoniae* (ATCC 10031), *Bacillus subtilis* (ATCC 10400), and Methicillin-resistant

staphylococcus aureus (MRSA) were employed in the investigation (ATCC 33519). *Candida albicans* fungal cultures were utilized as target organisms (ATCC 6538). Using the Agar Well diffusion method, the antibacterial properties of actinobacterial isolate extracts were investigated as (Magaldi *et al.*, 2004; Valgas *et al.*, 2007).

Wells were cut using sterile well borer on the agar surface seeded with the test organisms and 200 μ l of cell free supernatant from the fermentation broth of the isolate was added to each well aseptically. The plates were incubated at 37°C overnight for bacteria (Kirby Bauer method) and at 30°C for 72hrs for fungi (NCCLS M38- A2). Penicillin G and Nystatin were used as the positive controls for bacteria and fungi respectively. The

plates were examined for zone of inhibition around the well.

RESULTS

1. Isolation and Identification of bacterial strains

There is a diversity in the vegetation cover in the Al-Mamaria region in Riyadh, Kingdom of Saudi Arabia Figure (1), where eight different species of medicinal plants were collected and identified, as following (*Capparis spinose* family of *Capparaceae*, *Alhagi* family of *Fabaceae*, *Artemisia* family of *Asteraceae*, *Ducrosia* family of *Apiaceae*, *Artemisia monosperma* family of *Asteraceae*, *Cactus* family of *Cactaceae*, *Ziziphus* family of *Rhamnaceae*, and *Teucrium* family of *Lamiaceae*.



Figure 1: Map of Collection Sites

Twelve bacterial isolates were successfully isolated from plant samples, collected from the three different isolation sites located in Thumama region Riyadh Saudi Arabia.. The bacterial isolates were chosen

based on their morphological differences and were purified on the same isolation media used and then stored on slants at 4°C and in 20% glycerol at -20°C for further analysis (Figure 2).

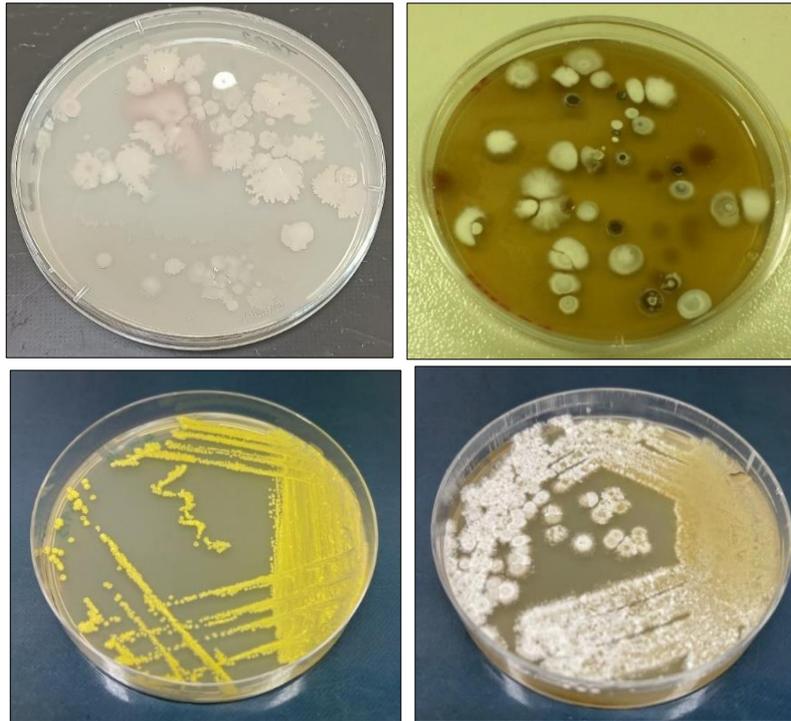


Figure 2: Isolation plates and de-replication of the selected strains in all tested sites of Thumama region used (ISP2) medium

(Isolate NA118) This strain was isolated from *Capparis spinosa*. The electron microscope image showing the spores chains of the mature aerial mycelium are short with smooth-surfaced spores (Figure 3). The

substrate mycelium didn't show fragmentation and specific structures like sporangia, synnemata or sclerotia were not observed.

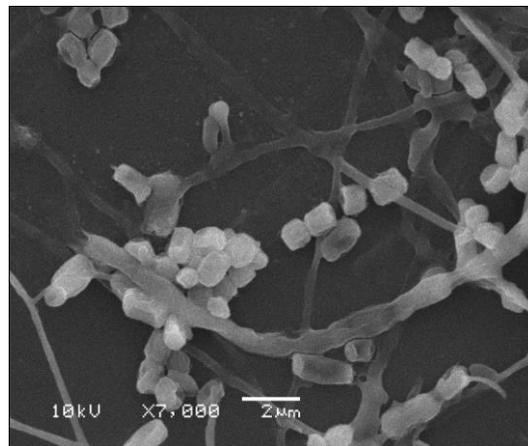


Figure 3: Electron micrograph of isolate NA118

The phylogenetic analysis of the 16S rRNA partial gene sequence of the 12 isolated strains was conducted as described in standard procedures from plant samples; the 12 bacterial isolates were belonging to 12 species in 7 genera. Eleven isolates belong to *Actinomycetota* (*Agromyces bauzanensis*, *Lentzea kentuckyensis*, *Streptomyces paromomycinus*, *Streptomyces caelestis*, *Rhodococcus gordoniae*, *Streptomyces lycii*, *Actinophytocola algeriensis*, *Streptomyces cellulosa*, *Aeromicrobium massiliense*,

Streptomyces roseicoloratus, *Streptomyces albidochromogenes*) and one isolate belong to *Bacillota* (*Bacillus siamensis*). A total of six bacterial strains were isolated and identified as belonging to the genera *Streptomyces* (6 species), One bacterial strain was identified as member of the genera *Agromyces* (1 species), One bacterial strain was identified as member of the genera *Bacillus* (1 species), *Lentzea* (1 species), *Actinophytocola* (1 species), *Aeromicrobium* (1 species), *Rhodococcus* (1 species), Table (1).

Table 1: Sequence similarity of isolated strains based on 16S rRNA sequences compared to the GenBank database

Sample Name	Most related similar strain						Score		Identities	
	Accession	Description	length	start	End	Cover age	Bit	E. Value	Match/ Total	PCt (%)
NA8	NZ_VBVX01000404.1	<i>Streptomyces albidochromogenes</i>	1460	2	1456	99	2615	0.0	1443/1456	99
NA103	NZ_BMMD01000048.1	<i>Agromyces bauzanensis</i>	1508	17	1497	100	2730	0.0	1480/1481	100
NA109	NZ_MUYM01000111.1	<i>Lentzea kentuckyensis</i>	1477	5	1461	99	2660	0.0	1452/1457	99
NA18	NZ_WHPN01000138.1	<i>Streptomyces lycii</i>	1484	1	1468	99	2693	0.0	1465/1468	99
NA182	NZ_JADBEA010000001.1	<i>Actinophytocola algeriensis</i>	1477	5	1463	100	2652	0.0	1452/1460	100
NA118	NZ_BHZD01000001.1	<i>Streptomyces paromomycinus</i>	1487	16	1486	100	2712	0.0	1470/1471	100
NA148	NZ_BMUR01000003.1	<i>Streptomyces caelestis</i>	1477	5	1465	100	2660	0.0	1454/1461	100
NA199	NZ_JOEV01000114.1	<i>Streptomyces cellulosa</i>	5343114	1758571	1757084	99	2741	0.0	1487/1488	100
NA20	NZ_HE978640.1	<i>Aeromicrobium massiliense</i>	1524	13	1478	99	2675	0.0	1461/1467	100
NA156	NZ_SDOY01000026.1	<i>Streptomyces roseicoloratus</i>	1470	1	1470	100	2708	0.0	1468/1470	100
NA111	NZ_AJVF01000043.1	<i>Bacillus siamensis</i>	1486	5	1460	99	2706	0.0	1486/1460	99
NA178	NZ_UGVI01000001.1	<i>Rhodococcus gordoniae</i>	1479	12	1469	100	2801	0.0	1474/1460	100

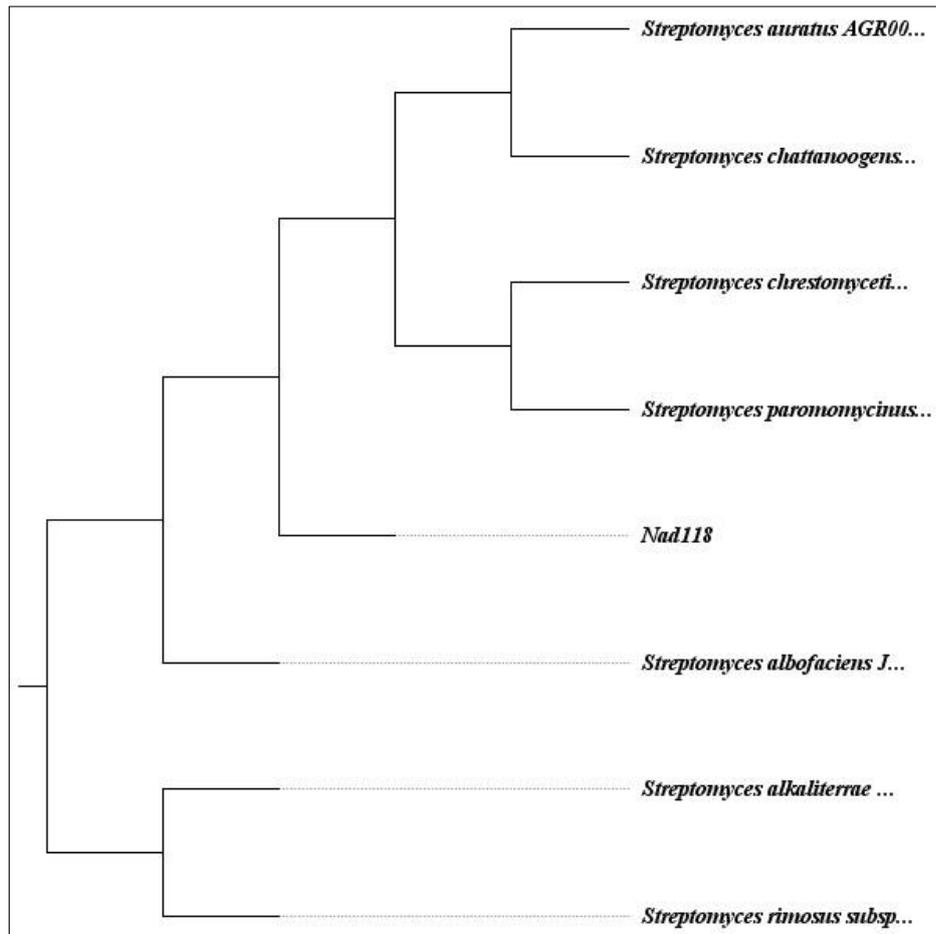


Figure 4: A neighbor joining tree of isolate (NA118) which show 100 % percentage genus *Streptomyces paromomycinus*

Preliminary comparison of the 16S rRNA sequence of isolate (NA118) against the GenBank database indicated that 100 % similarity percent of species *Streptomyces paromomycinus* accession no. NZ_BHZD01000001.1.

3. Screening the isolated actinobacterial strains for their antimicrobial activities

The antimicrobial activities of the selected 12 bacterial isolates were tested against some pathogenic Gram-positive, Gram-negative bacteria and yeasts. It was found that 10 isolates showed activities against the tested organisms as shown in Table (2) and Fig 6. It is obvious from the results that higher inhibition was recorded against Gram-positive bacteria and yeast while

Gram-negative bacteria were less inhibited. According to the size of the inhibition zone the biologically active strains can be divided into three groups as shown in Table (2):

Group 1: Slightly active strains which gave antimicrobial activities with inhibition zones from 10 to 20 mm .

Group 2: Moderately active strains which gave antimicrobial activities with inhibition zones ranging from 21 to 30 mm

Group 3: Highly active group which contains organisms that have the ability to inhibit the growth of the tested strains with inhibition zones more than 30 mm (Table 2, Figure 6)

Table 2: The antimicrobial activities of the biologically active bacterial isolates expressed as inhibition zones of growth against the used test organisms in mm

Samples number	<i>B. sub</i>	<i>Klebsiella</i>	MRSA	<i>C. alb</i>
NA 8	++	-	-	++
NA 103	+	-	-	-
NA 109	++	-	-	+
NA 18	++	-	-	+
NA 182	++	++	-	+
NA 118	+++	+++	-	++
NA 148	-	++	-	+
NA 199	-	+	-	-
NA 20	+	+	-	+
NA 156	+	-	-	-

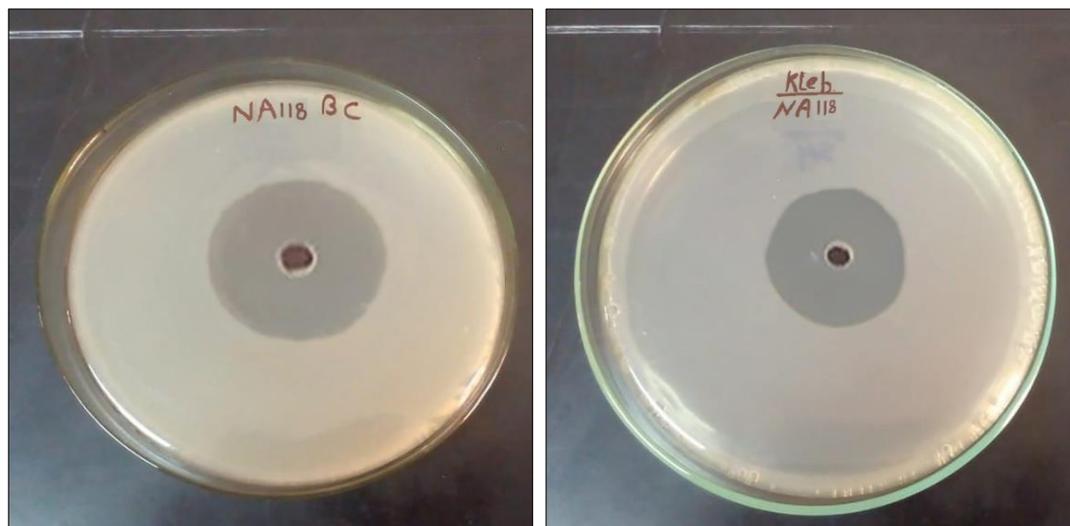


Figure 6: Inhibition zones of selected bacterial isolate NA118 against *Klebsiella pneumoniae*, *Bacillus subtilis*

4. Metabolites profiling

Essential and non-essential amino acids profile in the actinobacterial isolates were investigated Figure (7). Actinobacterial strains showed differential levels of amino acids, for instance isolate NA8 only accumulated the amino acids aspartate and tyrosine. Isolate NA118 showed the highest increases in valine, proline,

ornithine, leucine and methionine among all the other isolates. Isolate NA8 showed the highest level of beta-isorenieratene. The different isolates vary in their capacity to produce IAA and siderophore, whereas the highest values recorded for isolates number NA118, NA182 and NA18.

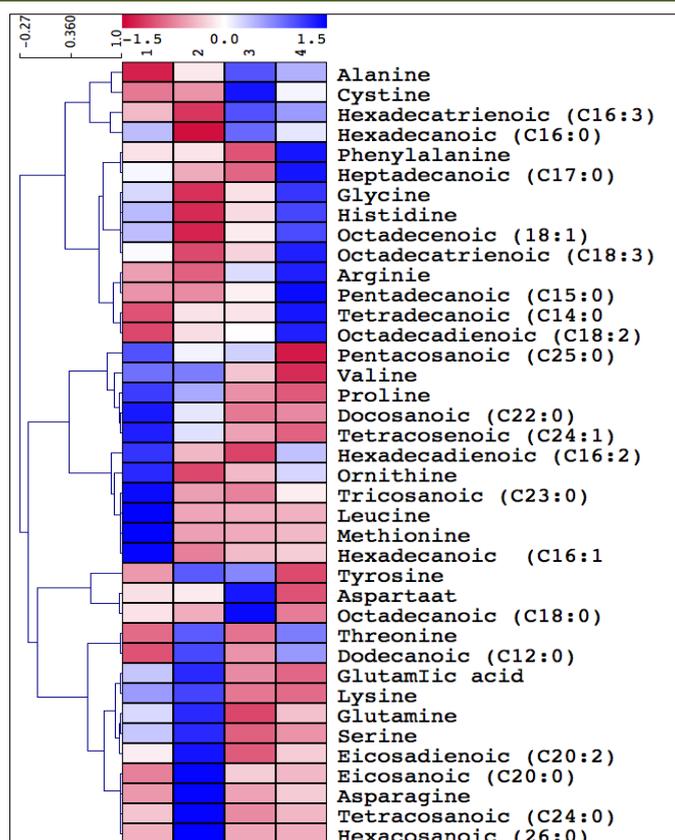


Figure 7: The Primary metabolites for the selected Actinobacteria

DISCUSSION

Generally, the symbiosis of endophytic bacteria and plants has a greater probability of producing chemical compounds than bacteria which live freely (Mearns-Spragg *et al.*, 1998). In this study we select total 12 Isolates belong to four phyla: Actinomycetota and Bacillota (Firmicutes) have been isolated from different plants (*Aerva javanica*, *Atractylis carduus*, *Calotropis procera*, *Capparis spinosa*, *Chrozophora oblongifolia*, *Cichorium Pumilum*, *Fagonia indica*, *Helianthemum lippii*, *Lycium Shawii*, *Malva Parviflora*).

Our 12 isolates purified by streaking method on the same isolation medium (Fig 2) based on dissimilarity in the color and shape of the colonies and then stored on slants at 4°C and in 20% glycerol at -20°C the rest of isolates stored for further analysis. 11 isolates belong to Actinomycetota (NA103 *Agromyces bauzanensis*, NA109 *Lentzea kentuckyensis*, NA118 *Streptomyces paromomycinus*, NA148 *Streptomyces caelestis*, NA178 *Rhodococcus gordoniae*, NA18 *Streptomyces lycii*, NA182 *Actinophytocola algeriensis*, NA199 *Streptomyces cellulosa*, NA20 *Aeromicrobium massiliense*, NA156 *Streptomyces roseicoloratus*, NA8 *Streptomyces albidochromogenes*), Only One isolate belongs to Bacillota (*Bacillus siamensis*).

Actinobacterial strains showed differential levels of amino acids, for instance isolate NA8 only accumulated the amino acids aspartate and tyrosine. Isolate NA118 showed the highest increases in valine,

proline, ornithine, leucine and methionine among all the other isolates. However, isolate NA 182 contained the highest level of phenylalanine, glycine, histidine, arginine. The actinomycetes have been shown to produce and excrete some amino acids like glutamic acid, alanine, valine and lysine (Rozycki *et al.*, 1986). Isolate NA18 showed the highest level of threonine, glutamic acid, lysine, glutamine, serine and asparagine. Serine appears to be an important determinant of hydrophobicity because of its compact size and borderline hydrophobic– hydrophilic character (Dennis and Shimmin 1997). Metallic nanoparticles such as zinc oxide nanoparticles and MgO nano-flowers showed high antibacterial activity against bacterial leaf blight disease (Ogunyemi *et al.*, 2019; Abdallah *et al.*, 2019).

Streptomyces paromomycinus NA118 exhibited antimicrobial activity against the three tested microbes *Klebsiella pneumoniae*, *Bacillus subtilis* and *Candida albicans* which is partially consistent with (Kaur *et al.*, 2019) which showed that the antimicrobial activity of *Streptomyces paromomycinus* has been evaluated against various bacterial and fungal pathogens, with the highest activity observed against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* (Kaur *et al.*, 2019). Beside, the *S. paromomycinus* extract exhibited a rapid bactericidal effect against *S. aureus* and *E. coli*. Also *Actinophytocola algeriensis* NA182 exhibited antimicrobial activity against 3 tested microbes *Klebsiella pneumoniae*, *Bacillus subtilis* and *Candida*

albicans which is in partially in agreement with previous report by (Boubetra & Sabaou, 2021) who identified antimicrobial of polyketide-derived compound produced by *A. algeriensis*, that has shown antimicrobial activity against a variety of bacteria and fungi, including *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and *Aspergillus fumigatus*.

CONCLUSION

The findings we got from the symbiotic relationship between Collected plants and their associated bacteria in this study indicating that there is microbial diversity. Few studies performed in this field. therefore, Further studies needs to be performed in which we can isolate and identify various actinobacterial species using different media beside it could have interesting biologically active metabolites. Detection of interesting primary and secondary metabolites from actinobacterial strains have been taken place in this study including (amino acids. Fatty acids. Alkaloids, flavonoids, carotenoids) that have nutritional importance, as source of energy and antioxidant activity.

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المخلص العربي

تبرز النباتات كمصدر حيوي للتنوع الميكروبي ، وبالتالي كمصدر ايضا للمركبات الايضية الثانوية النشطة بيولوجيا. تهدف هذه الدراسة إلى تمييز المركبات الأيضية وتقييم النشاطات الضد ميكروبية للأكتينوبكتيريا بمنطقة المرتبطة بالنباتات الصحراوية المجموعة من صحراء التمامة Agar well diffusion. تم تقييم النشاطات الضد ميكروبية باستخدام طريقة وكذلك تم تمييز المركبات الايضية باستخدام جهاز HPLC مطياف الكتلة كروماتوغرافيا السوائل عالي الكفاءة . أظهرت العديد من مستخلصات GC-MS كروماتوغرافيا الغاز الاكتينوبكتيريا نشاطًا مضادًا للميكروبات ضد العديد من الميكروبات نشاطًا مضادًا N182 و NA118 المختبرة ، أظهرت عزلات *Klebsiella* للميكروبات ضد الثلاثة المختبرة *pneumoniae* و *Bacillus subtilis* و *Candida albicans* . أظهرت سلالات الاكتينوبكتيريا احتوائها على مستويات متباينة من أعلى زيادة في الفالين ، NA118 الأحماض الأمينية ، وأظهرت العزلة البرولين ، الأورنيثين ، الليوسين. تم تسجيل أعلى مستويات من سداسي . كان NA8 لعزل (hexadecanoic (C16: 0) و C16: 3 الحلقات) غنياً بالعديد من مركبات الفينول والفلانويد. تم تسجيل NA182 عزل محتويات عالية من بيتا توكوفيرول و دلتا توكوفيرول وجاما توكوفيرول وأخيراً تم تقييم الخصائص الدوائية NA18 و NA118 العزل للمستخلصات البكتيرية. كشفت الاختبارات المضادة للبكتيريا أن 10 من أصل 12 مستخلصًا بكتيريًا كان لها أنشطة مضادة للميكروبات ضد واحد لذلك ، يجب إجراء . على الأقل من الكائنات الحية الدقيقة التي تم فحصها مزيد من الدراسات التي يمكننا من خلالها عزل وتحديد أنواع الاكتينوبكتيريا المختلفة باستخدام بيانات مختلفة بجانب انه يمكن أن تحتوي على مستخلصات متنوعة وجديدة نشطة بيولوجيًا مثيرة للاهتمام.