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Production of granticin by *Streptomyces spp* isolated from Iraqi soil samples

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Abstract: *The differentiates Streptomyces species from other kinds of bacteria is that they are able to produce substances such as granticin, actinorhodin, and medermycin.These pigments are known as acid-base indicators because of their properties. For example, graniticin turns red in acidic environments and purple in alkaline ones. Finally, medermycin has an acidic yellow color and an alkaline brown color. A few pH-sensitive pigments have antibacterial qualities, including granaticin, undecylprodigiosin, and actinorhodin. In this study, 103 soil samples were collected from seven Iraqi provinces. Ten isolates were obtained that inhibit pathogenic bacteria. The Streptomyces isolates are identified by microscopy and morphology. These isolates were diagnosed genetically through the 16s rRNA gene. Five isolates were sent for sequence analysis. After that, the Streptomyces isolates that produced granticin were searched for by means of by changing the color of the producer isolate from red to violet when adding the base NaOH and genetically through the orf-gra 11 gene that encoding to a dTDP-glucose dehydratase enzyme granticin (500bp) by conventional PCR ,one isolate was obtained that produced granticin Streptomyces RS18. By examining the inhibition activity of the isolates, the isolate RS18 showed effective inhibition against gram -positive bacteria, and there was no activity for gram-negative bacteria. The aim of this study is the isolation and identification of Streptomyces spp . Detection of granticin gene gra 11 that encode for granticin production and their antibacterial activity against pathogenic bacteria. Detection of granticin production among streptomyces isolates. Further chemical characterization of the local iraqi granticin is very usefull in its pharmaceutical companies.*

Keywords: *Streptomyces, granticin, antibacterial spectrum*

1.Introduction

Actinomycetes are omnipresent and often in very large numbers existing in almost every habitat from the poles to the equator. The majority of actinomycetes are saprophytes and some are parasites or endophytes. Since they are physiologically and nutritionally distinct,

are adaptable to a variety of environmental conditions. This group is able to degrade all types of organic compounds making them ubiquitous in nature. They occur in multiplicity of natural and man-made habitats such as aquatic and terrestrial ecosystems (Kurtböke, 2017). The synthesis and production of a variety of bioactive compounds by the Streptomyces , such as antibiotics, antifungals, and antivirals, is well known (Tomaseto *et al*., 2020) **.**

Streptomyces comprises a diverse group of filamentous bacteria belonging to the family Streptomycetaceae within the order Actinomycetales. With a distribution in both soil and water environments, this genus encompasses over 500 distinct species. Numerous organisms play a crucial role in organic matter breakdown inside soil(Britannica, 2020). *Streptomyces* have a stationary growth pattern like plants, as they lack motility and instead rely on the production of spores for dispersal, analogous to the dispersal mechanism observed in seeds. The passive nature of these bacteria renders them relatively susceptible to predation, leading to the hypothesis that Streptomyces produce antibiotics as a defensive mechanism against motile microorganisms in the soil (Lee *et al*., 2020).

Streptomyces , a type of filamentous soil bacteria, holds significant medical and economic value due to its Gram-positive nature and capacity to synthesize diverse bioactive compounds that have practical use in human health and agriculture(Shikura *et al*., 2021). To compare bacterial genetic materials for identification, genes present in all the bacteria such as *rRNA* genes are required. On the other hand these genes should be highly conserved in the particular species. Since the 16S rRNA gene is the most highly stable among the rRNA genes, its sequencing has become the accepted norm for bacterial species identification and taxonomic categorization (Bouchet *et al.*, 2008).

Compounds production is a characteristic that sets members of the Streptomyces genus apart such as actinorhodin, granticin, and medermycin.

The pigments mentioned above possess characteristics that classify them as acid-base indicators. For instance, actinorhodin exhibits a red hue in acidic environments and a blue hue in alkaline environments.

Similarly, graniticn displays a red color in acidic settings and a purple color in alkaline situations. Lastly, medermycin appears yellow in acidic conditions and brown in alkaline conditions. The presence of pH-sensitive pigments or litmus-like pigments was discovered in Streptomyces coelicolor (Muller). Certain pH-sensitive pigments, such as actinorhodin, granaticin, and undecylprodigiosin, exhibit antibacterial properties. Additionally, some of these pigments have been found to possess antitumor activity, as exemplified by granaticin (Taher *et al*., 2020).

Granaticins exert their antibacterial effects by disrupting the process of tRNA aminoacylation, hence impeding the synthesis of proteins and RNAs. Granaticin, a benzoisochromanequinone (BIQ) family member, has garnered significant interest from chemical and biochemical researchers owing to its distinctive sugar attachment and the inverse stereochemistry of its pyran ring (Iwasaki & Ōmura, 2007) . The aim of this study is the isolation and identification of *Streptomyces spp* . Detection of granticin gene *gra 11* that encode for granticin production and their antibacterial activity against pathogenic bacteria. Detection of granticin production among *Streptomyces* isolates.

2.Methodology

Sample collection:

All samples were collected randomly depending on farming and non-farming soil from Seven central districts Baghdad, Diwaniyah, Nasiriyah, Musil, Kut, Najaf, and Hella, summarized in Table from 11/ 2021 to

4/2022. They were collected from the surface of the soil, 10 cm depth**.** The total collected samples were 103 soil samples. The soils were placed inside unused sterile nylon bags and transferred to the laboratory. After taking the pieces to the laboratory, the samples were dried from the excess moisture, then the soil was crushed to get rid of the large lumps, and then the soil was sifted to get rid of the stones and tree branches mixed with the soil.

Pretreatment of soil

Following four days of drying at 37 ˚C in a thermal oven with calcium carbonate added at a ratio of one:ten w/w, samples of soil were suspended in a sterile destiled water by dividing (one) g of soil to (nine) ml . From the solution in a sterile test tube, a dilution (10^{-1}) was obtained, and (one) ml was transferred from it to a second sterile test tube containing (nine) ml of said solution (Kutzner, 1981)**.**

The samples were subjected to a 10^{-2} dilution and transferred into test tubes. These test tubes were placed in a water bath set at 45°C for 16 hours. This process aimed to facilitate the separation of spores from vegetative cells. Following this, the dilutions were inoculated onto the surface of Actinomycete Isolation Agar plate. The plates were incubated at 28°C for 7 to 14 days(Ceylan *et al*., 2008).

Pathogenic bacteria

Five isolates, including Gram positive [*Staphylococcus aureus*]and Gram negative [*E.coli ,Klepsiella pneumonia , Pseudomonas aeruginosa and Acinetobacter baumannii*] were used to determine the antibacterial activity of Streptomyces . These microorganisms were obtained from Ibn Albaladi hospital. The diagnosis of these isolates was confirmed by conducting a number of diagnostic tests and using the necessary culture media for diagnosis

Identification of Streptomyces isolates

Bacterial isolates were diagnosed based on their phenotypic characteristics [colony shape, secretion of pigments, color of aerial and ground mycelium], then the characteristics of bacterial cells were studied microscopically after staining with gram stain, which included (the shape of blackboards,hyphae and their ability to stain with gram stain) according to what was stated in (Kavitha & Vijayalakshmi, 2007)

Identification of selected Streptomyces isolates

Molecular Characterization.

The process of isolating genomic DNA involved the utilization of the bacterial genomic DNA fast extraction kit manufactured by Norgen in Canada. Subsequently, the bacterial 16S rRNA was amplified by applying universal primers as specified in Table 1. PCR amplifications were conducted using a Trio PCR System (Biometra, Germany). This procedure involved the addition of 12.5 µl of OneTaq (NEB®) mastermix, 3 µl of DNA sample, 1 µl of 10 pmol/µl primer for each primer, and 7.5 µl of free-nuclease water. The PCR reaction was performed using the optimum conditions specified in Table 2 for the target gene. Agarose gel electrophoresis was employed after PCR amplification to validate the existence of PCR product . The success of PCR was entirely reliant on the presence of extracted DNA as a crucial criterion (Sambrook & Fritsch, 1989)**.**

Table 1: Primer sequence and Product size of genes of *Streptomyces* bacteria(Algafari et al., 2020).

Primer Name	Sequence 5`-3`	Annealing Temp. (°C)	Product Size (bp)
Forward 16s	GATTAGTGGCGAACGGGTGA	55	472
Reverse 16s	CCTACGAGCTCTTTACGCCC	55	472

Molecular Detection of 16S rRNA gene

Table 2 : PCR cycling conditions for 16S rRNA gene and ORF-gra 11gene amplification **.**

PCR Stage	Temperature	Time sec cycle
Initial Denaturation	94 ºC	5 mins 1
Denaturation	94 ºC	30 sec.
Annealing	57 ºC	38x
Extension	72 ºC	55 sec. 45 sec.
Final Extension	72 ºC	7 mins. 1

PCR product (*16s rRNA* and *gra-orf 11* Sequencing

Five ***16s rRNA*** **and one *gra-orf 11*** of PCR products was sent for Sanger sequencing using ABI3730XL, automated DNA sequencer, by Macrogen Corporation – Korea.

Characterization of *Streptomyces* Isolates

Primary screening of suspected *Streptomyces*

The suspected colonies of genus *Streptomyces* were transfered from the first isolation step (mixed culture) in to fresh Yeast extract _Malt extract agar media (ISP2)to obtain pure culture .In this step, isolates were selected based on its red pigment production (granticin) on ISP2 media . The selected pigment producing colonies were then incubated at 30℃ for 7 days and kept at 4 ˚C for further study (Bernard, 2007). At the same regard(Taher *et al*., 2020) found that the *Streptomyces* IQ45 produce a pH-sensitive pigment (actinorhodin -like substance) at which the red colonies convert into blue color after fuming over ammonia as a qualtitive detection of actinorhodin production by this isolate .

Secondary screening of *Streptomyces* isolates

The Secondary screening was performed via evaluating the antibacterial potential of pigments produced from the selected Streptomyces isolates by agar disc diffusion method (Egorov, 1985)against five indicator strains as follows ;{*staphylococcus aureus ,E.coli ,klebsella kneumonni ,pseudomonase aurigenosa ,Acinetobacter baumannii }*

Screening for granticin produces

The resulting pure culture of suspected granticin-producing *Streptomyces* colonies were tested for granticin production by two methods:-

Qualtatively

The ability of ten *Streptomyces* isolates for producing of pH -sensitive antibiotic (granticin) was detected by the conversion of red substrate pigment into violet color after adding of drops of NaOH (0.1N) on the surface of ISP2 dishes filled with *Streptomyces* colonies grown for 5 days at 28 C which have substrate mycelia with red colour ,changing to violet color represents a positive result (Snipes *et al*., 1979).

Genetically

The granticin production controlled by a cluster of genes ,in our study a primer for one granticin gene(gra) no 11 was assigned and the detailes was allustrated as in table(3)

Table 3: The primer of gra 11 gene

Primer Name	Sequence 5`-3`	Annealing Temp. (°C)	Product Size (bp)
Forward	CTGTGTCGGATTCACCGCAC	55	500
Reverse	GACAGGTTGTAGCCGAGCAG	55	500

Results

Soil sample collection

Ten isolates were tested to produce granticin, and only one isolate produced granticin while the other nine have another bioactive compounds production ability to produce these compounds as in table (4) .

Table (4) :-Detection of granticin production by *Streptomyces* sp.

Streptomyces isolate	**Granticin production**
RS1	-
RS3	-
RS5	-
RS10	-
RS18	+
RQ23	-
RS38	-
RS42	-
RS44	-

(+) =Granticin production
(-)=No granticin production

Suspected *Streptomyces* colonies were further subcultured on ISP2 agar carefully to obtain pure isolates. All *Streptomyces* isolates were subjected to screening program based on their ability for granticin production as detailed in the following sections.

Cultural Characterization of Streptomyces Isolates

The morphological and microscopic qualities of the sample were assessed through visual inspection using light microscopy. Additionally, the Gram-stain properties of the sample were examined, as depicted in Fig. 1. The findings indicated the presence of 21 isolates associated with *Streptomyces spp*. Our results showed that all these *Streptomyces* isolates were a gram- positive bacteria.

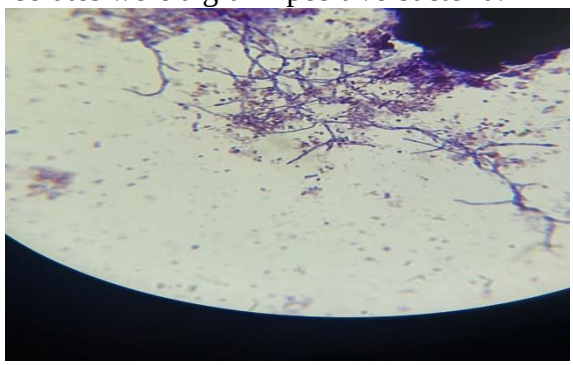


Fig. 1. The macroscopically characteristics of Local *Streptomyces Spp(Rs18)*. were observed with Gram's stain under **10x** and oil-immersion **(1000x).**

Morphological and cultural characteristics of *Streptomyces* isolates.

The features of *Streptomyces* isolates showed that they were having different mycelium shapes , different substrates color and differed in presence or no soluble pigments as show described in Fig .2.

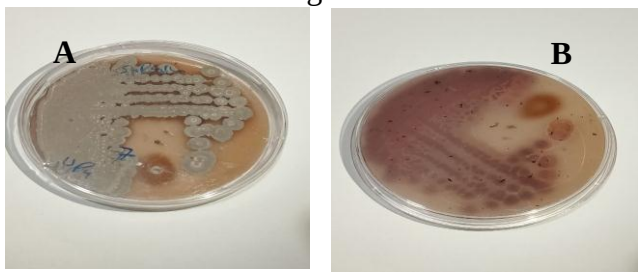


Fig.2 . *streptomyces sp*.RS18 on ISP4 media
A-aerial mycelium of *streptomyces* RS18
B- substrate mycelium of *streptomyces* RS18

Primary screening of suspected *Streptomyces*

Results showed that only one isolate could produce violet color as,tested by exposure to alkaline conditions (drops of NaOH) as in Fig. 3 . That it might be possible to suggest that *Streptomyces* (RS.18) is an granticine _ like substance producer isolate (Pyrek *et al*., 1977).

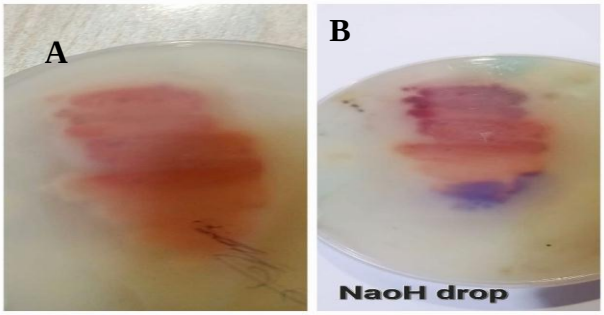


Fig.3. Qualtative detection of granticin production by Streptomyces RS18.
A-colour of substrate mycelium before adding Na oH
B- Change the color of the substrate to violet after adding NaoH

Secondary screening of Bioactive producers *Streptomyces* isolates

Secondary screening was performed by evaluating the antibacterial potential of the ten selected *Streptomyces* isolates by agar plug diffusion method against five indicator strains *(Staphylococcus aureus, E.coli , Klebsiella pneumonia, Pseudomonas aeruginosa* and *Acinetobacter baumanii)* . Our results showed that the RS18 isolate (granticin producer) showed a little inhibitory effect on gram positive bacteria (*Staph aureus*)only with inhibition zone diameter of 10 mm in diameter as in table (5) and there was no activity against other gram negative tested pathogenic bacteria as in figure .4.

Table 5: Inhibitory activity of ten selected *Streptomyces* isolates against pathogenic bacteria

Streptomyces isolate	Zone inhibition (mm)				
	Staphylococcus aureus	*E.coli*	*Klepsella pneumonia*	*Pseudomonas aeruginosa*	*Acenitobacter baumanii*
RS1	9	10	10	9	11
RS3	11	11	10	12	13
RS5	15	17	14	13	16
RS10	15	16	17	12	12
RS18	10	0	0	0	0
RQ23	17	29	26	22	22
RS38	10	12	12	12	10
RS39	19	13	12	14	11
RS42	10	11	10	10	9
RS44	12	12	10	9	10

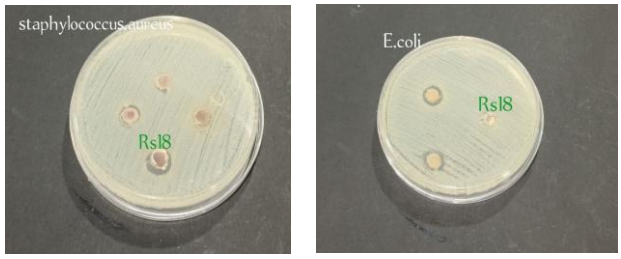


Fig.4. The inhibitory activity of Streptomyces isolates : RS18 against *Staph. Aureus=(10 mm)* .and *E.coli =(0).*

Molecular detection for granticin production

The granticin production controlled by acluster of genes on the bacterial chromosome (Deng & Zhu 2011) .In our study ,only one granticin (gra11)gene was assigned and detected for Streptomyces .RS18 . After detection of the *Streptomyces spp* by genetic conventional PCR and sequencing, the next step was the detection of *gra- orf 11* . The main present study objectives was to found the gra- orf 11encoded to dTDP-glucose 4, 6-dehydratase enzyme granaticin.

Our results showed that only *Streptomyces* RS18 had this gene of (500bp) among the five tested *Streptomyces* isolates which confirmed its ability for ganticin production as in Figure 5.

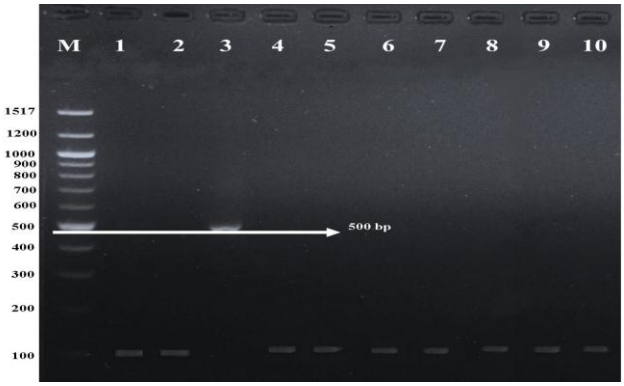


Fig.5. Conventional PCR products of granticin gene gra- orf 11 by five Streptomyces isolates (1-10) number3 represent Streptomyces RS18 isolate . The amplicons size was approximately 500bp. The amplicons were run on agarose ge l 2% and visualized with transilluminator, M: Marker (ladder) ranged 100- 1517bp. The using

dye was using Red safe dye. Run the gel was 80V at 80min.

Consequently, one local *Streptomyces* sp isolate *gra* gene were analyzed and compared with a reference strain available in the Genbank database of National Center Biotechnology Information (NCBI). After using Basic Local Alignment Search Tool (BLASTN) program which is available at the NCBI, the result of sequencing appeared 100% compatibility for isolate. The identity of nucleotide sequence to the ***gra-orf 11*** gene for isolate compared with China under accession number [MN861990.1] as shown in Fig.6.

Isolate was matched with *Streptomyces* sp isolate strain QHH-9511 aromatic polyketide biosynthetic gene cluster, complete sequence. Sequence ID: MN861990.1 Length: 40897

Number of Matches: 1 Range 1: 25745 to 25996.

Score	Expect	Identities	Gaps	Strand
466 bits(252)	1e-126	252/252(100%)	0/252(0%)	Plus/Plus

```
CDS: Putative 1      83    * S A P A R W F D P H D R Y W D V V A G
Query                1     TCAGGAGGCCGGGGCGCGCCAGAAGTCGGGGTGGTCGCGGTACCAGTCCACGACCGCCCC  60
Sbjct                25745 ..............................................................  25804
CDS:dTDP-glucose 4,6 83      S A P A R W F D P H D R Y W D V V A G

CDS: Putative 1      64    L G S E I S W R P A Y G L E E R I K S D
Query                61    CAGCCCGCTCTCGATCGACCAGCGGGGCGCGTAGCCCAGCTCCTCGCGGATCTTGGAGTC  120
Sbjct                25805 ..............................................................  25864
CDS:dTDP-glucose 4,6 64    L G S E I S W R P A Y G L E E R I K S D

CDS: Putative 1      44    D I A Y R F D H G L R D E V H R V M D W
Query                121   GTCGATCGCGTAGCGGAAGTCGTGCCCGAGGCGGTCCTCGACATGGCGCACCATGTCCCA  180
Sbjct                25865 ..............................................................  25924
CDS:dTDP-glucose 4,6 44    D I A Y R F D H G L R D E V H R V M D W

CDS: Putative 1      24    D A G L L D L L R A T M E R N S M G S G
Query                181   GTCCGCCCCCAGGAGGTCCAGCAGGCGGGCGGTCATCTCGCGGTTGCTCATACCGCTGCC  240
Sbjct                25925 ..............................................................  25984
CDS:dTDP-glucose 4,6 24    D A G L L D L L R A T M E R N S M G S G

CDS: Putative 1      4     G L R M
CDS: Putative 2            *
Query                241   GCCGAGCCTCAT  252
Sbjct                25985 ............  25996
CDS:dTDP-glucose 4,6 4     G L R M
```

Fig.6. Sequences of *Streptomyces* sp isolate *gra* gene with *Streptomyces* sp strain QHH-9511. No found differences in the nucleotides of this study query and the subject

Phylogenetic analysis

Streptomyces spp sequences from the ***gra-ORF11* gene** studied in one Streptomyces sp isolates are displayed in the phylogenetic tree constructed by the neighbour-joining method.

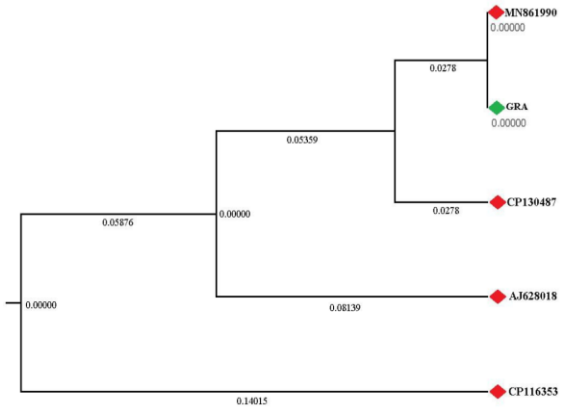


Fig. 7. Phylogenetic tree including one local *Streptomyces* sp isolates sequences in the *gra-ORF11 gene* and 4 isolates from China, Vietnam, and Korean, clustering based on neighbor-joining maximum composite likelihood method by using MEGA X program version 11.0.13

Molecular identification of Streptomyces isolates

Amplification of 16s rRNA gene by conventional PCR One of the main present study objectives is to determine the Streptomyces spp by amplifying specific primers design were used to amplify 16s rRNA (472bp) in 10 sample by using conventional PCR by specific primer design .Only 10 samples from 20 of 16s rRNA using conventional PCR, the volume of amplicons was approximately 472bp, confirmed by comparing their molecular weight with the 100 bp DNA ladder as shown in Fig.8.

Our results confirmed that all the five sequenced *Streptomyces* isolates have the same 16s rRNA (472bp) and finally allof them were related to the same classified speciese (Streptomyces sp 100%) after analyzed and compared with a refrence strain available in the Genbank database of National Center

Biotechnology Information (NCBI) as explained in the following sections .

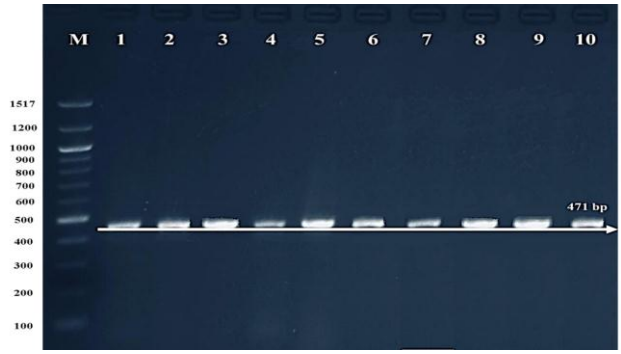
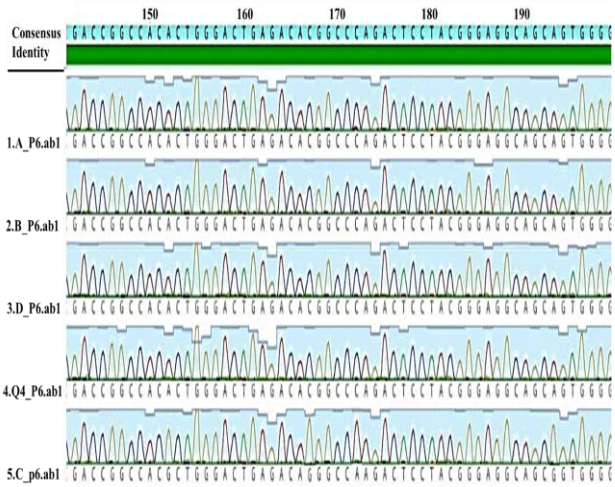


Fig.8. Five samples (1, 2, 3, 4, and 10) were amplified 16s rRNA by conventional PCR The amplicons size was approximately 472bp. The amplicons were run on agarose gel 2 % and visualized with transilluminator, M: Marker (ladder) ranged 100-1517bp. The using dye was using Red safe dye . Run the gel was 80 V at 80 min.

To emphasis *Streptomyces* spp diagnosis and classification in the current study, five positive samples were sequenced after amplified 472bp of *16s rRNA* gene by conventional PCR. Nucleic acid sequencing was conducted to emphasize their specificity and introduce the ultimate means to detect the



Streptomyces spp species and strain.
After received the sequences from Macrogene company three format editing the sequence by using Geneious program version 2023.2. show Fig.9.

Fig.9. The sequence of five PCR products to local *Streptomyces* spp isolates *16s rRNA* gene after edited by using Geneious program version 2023.2

Comparison nucleotide sequences from the *16s rRNA* gene of five Iraqi *Streptomyces* spp isolates from Baghdad province all this isolate analyzed by (NCBI program through BLASTN, and using Multiple alignment by Geneious program vision 2023. 2 as shown in Fig.10, with different reference strains showed that 100% of local *Streptomyces* spp isolates belonged to *Streptomyces* sp. The identity in nucleotide sequence 100% for 4 isolates and 96.33% for 1 isolate comparison China isolate under accession number [OR143870.1].

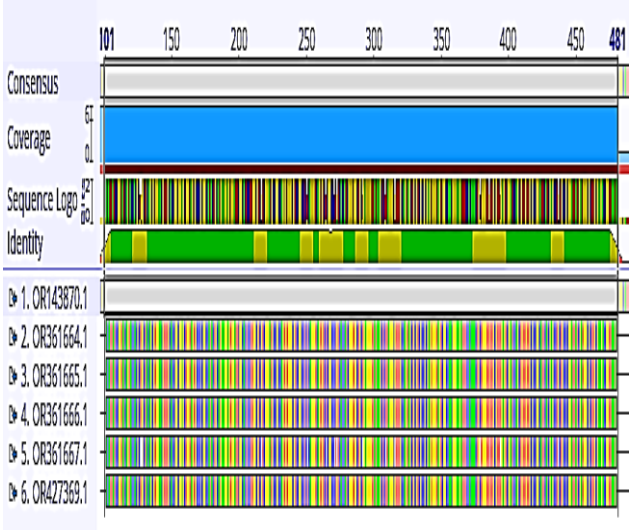


Fig.10. Multiple alignment of the nucleotide sequences from the *16s rRNA* gene of 5 Iraqi *Streptomyces* sp to [OR143870.1] isolate by Geneious program vision 2023.2.

Submission 5 sequences isolates of the *16s rRNA* gene from *Streptomyces* sp in this study in Genebank (NCBI) under accession numbers [ID: OR361664.1, OR361665.1, OR361666.1,

OR361667.1, and OR427369.1]. When comparing the results of this study with studies such as.). (Aliero et al., 2018).

Phylogenetic analysis

Streptomyces spp sequences from the *16s rRNA* studied in all 5 *Streptomyces* sp isolates are displayed in the phylogenetic tree constructed by the neighbour-joining method.

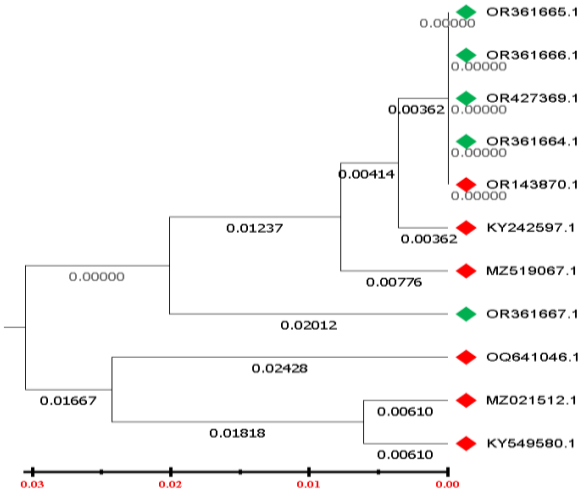


Fig. 11. Phylogenetic tree including 5 local *Streptomyces* sp isolates sequences in the *16s rRNA* and 6 isolates from China, France, Iran, USA, Turkey and Brazil, clustering based on neighbor-joining maximum composite likelihood method by using MEGA X program version 11.0.13

Discussion

Ten isolates were tested to produce granticin, and only one isolate produced granticin while the other nine have another bioactive compounds production applity to produce these compounds . In order to isolate Streptomyces, it was noted that treating the soil with calcium carbonate (CaCO3) and drying it at a temperature of 37°C for 4 days has a significant role in increasing the numbers of Streptomyces bacteria in the initial isolation, because drying the soil leads to a reduction in the numbers of vegetative bacteria on the one hand, and adding calcium carbonate leads to an increase in the value of pH, which causes limiting the growth of most fungi on the other hand, thus promoting the growth of filamentous bacteria (Näher *et al*., 1997). This is similar to what was stated in a number of studies (Ceylan *et al*., 2008), noted that adding calcium carbonate to soil samples gives better results in isolation.

The morphological and microscopic qualities of the sample were assessed through visual inspection using light microscopy. Additionally, the Gram-stain properties of the sample were examined, as depicted in Fig. 1. The findings indicated the presence of 10 isolates associated with Streptomyces spp. The categorization and differentiation of International Streptomyces Project type 2 (ISP2) were based on observing many morphological features. The hue of mature sporulating aerial mycelium was seen on ISP2 plates. The aerial mass was categorized based on Bergey's manual of systematic bacteriology into various color classifications, including gray, white, red, yellow, and violet. Also by substrate mycelium.

The members belonging to the genus Streptomyces are renowned for their remarkable capacity to synthesize a diverse range of secondary metabolites. Several of these components are utilized as antibiotics, Ten Streptomyces isolates were selected for possible production of pH _sensitive pigments (granticin).

Results showed that only one isolate could produce violet color as,tested by exposure to alkaline conditions (drops of NaOH) as in Fig. 3 . That it might be possible to suggest that Streptomyces (RS.18) is an granticine _ like substance producer isolate (Pyrek *et al*., 1977).

Secondary screening was performed by evaluating the antibacterial potential of the ten selected Streptomyces isolates by agar plug diffusion method against five indicator strains *(Staphylococcus aureus, E.coli* ,

Klebsiella pneumonia, Pseudomonas aeruginosa and Acinetobacter baumanii) . Our results showed that the RS18 isolate (granticin producer) showed a little inhibitory effect on gram positive bacteria (Staph.aureus)only and there was no activity against other gram negative tested pathogenic bacteria . This is similar to what was mentioned in a number of studies, as Gilpin and his group (1988) noted.

Grantacin has activity against Gram-positive bacteria. While the nine isolates showed different activity against bacteria positive and negative for gram stain. The observed variation in antibacterial activity among the test bacteria can be attributed to disparities in the structure and composition of their respective cell walls. Gram-positive bacteria have peptidoglycan polymers near the cell surface, facilitating the ease of penetration by antibacterial compounds. In contrast, Gram-negative bacteria possess an outer membrane composed of lipopolysaccharides, a barrier against hydrophobic and hydrophilic compounds of specific molecular weights (Soares *et al*., 2012).

As a result for the nature of cell wall of G-ve bacteria which have Lipopolysaccharide (LPS) and phospholipid membrane that prevent the enterance of many antibiotics to inside cell and the ability of G-ve bacteria to less the permeability of cell membrane by decrease porins and increase efflux pumps (Friedman *et al*., 2016).

Conclusions

The following conclusions can be made based on the findings of the current study:The isolation of new indigenous Iraqi Streptomyces isolates with distinct inhibitory activity against pathogenic bacteria that make multiple additions to the current literature requires the constant screening of highly antibiotic-producing soil habititous.Only one isolate *Streptomyces sp*. RS18, is capable to producing granticin, which has inhibitory effects on *Staph aureus*. Further physichochemical character-rization definatelly is requied and also its invivo applications as a normal pharmaceutical products.Future study that includes knowledge of the effect on cells when used as an antibiotic on mice and thus its effect on cells when used as an antibiotic.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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