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# Molecular characterization of covalently closed circular plasmid DNA in local *Streptomyces* strains isolated from soil with detection of antibiotic production and antibacterial activity

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ABSTRACT

Various of *Streptomyces* species have two kinds of plasmids, circular plasmids (8 to 31 kb) and linear plasmids (12 to 1700 kb). Covalently closed circular (CCC) plasmids are profuse in the genus of *Streptomyces* and involved in production and resistance of antibiotics by genetic controlling. We collected fifty clinical soil samples from different regions in Al-Najaf Al-Ashraf province/Iraq. The samples included five from Al-Ghadeer Quarter, five from Al-Karama Quarter, 10 from Kufa University, five from Al-Ameer Quarter, four from Al-Forat Quarter, 10 from North Quarters and eleven from desert roads in Al-Najaf. Diluted samples were cultured on Yeast extract Malt extract (YEME) agar medium as a selective medium; then the presumptive *Streptomyces* colonies were subcultured on Tryptone Yeast extract (TYE) agar, then incubation at 37°C for 7 days. Seven biochemical tests for identification of *Streptomyces* isolates these are: Catalase test, Oxidase test, Urase test, Kligler Iron Agar test (KIA), Simmon's Citrate test, addition to MacConkey agar test and Mannitol Salt agar test. Five antibiotic discs were used for detection of antibiotic sensitivity of the *Streptomyces* isolates; these are: Tetracycline, Gentamycin, Vancomycin, Ampicillin, Erythromycin. The sensitivity of the antibiotics was observed by recorded the diameter of inhibition zone around the discs. Two test bacteria (*Staphylococcus aureus* and *E. coli*) were used for the determination of antibacterial activity. Plasmid isolation was done by the alkaline lysis method. This method is characterized by the rapid isolation of DNA from *Streptomyces*. Then, detection of Plasmid DNA occurred by using agarose gel electrophoresis.

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## INTRODUCTION

*Streptomyces* species are a group of prokaryotes represented in nature by the largest number and varieties among the family Streptomycetaceae on the basis of morphology and cell wall properties (Taddei, 2006). The genus *Streptomyces* are proposed by Waksman & Henrici (1943) as strictly aerobic, spore-forming actinomycetes, gram-positive rods with high guanine + cytosine contents in their DNA (70%) compared with other bacteria such as *E. coli* (50%) and a complex lifestyle. Two species of *Streptomyces* have been well studied: the first one that used in the production of antibiotics strep-

tomycin called *Streptomyces griseus*, the other one called *Streptomyces coelicolor* that used in genetic studies, in addition, they produce several secondary metabolites no less than four (Davies and Procópio, 1999). The genus *Streptomyces* are characterized by nonmotile, catalase-positive, reduce nitrate to nitrites, degrade adenine, esculin, casein, gelatin, hypoxanthine, starch, and L-tyrosine (Smaoui *et al.*, 2011).

*Streptomyces* are mycelial microorganisms which produce two kinds of branching mycelium, aerial mycelium and substrate mycelium, similar in appearance to the mycelium of some fungi (Al-Saadi *et al.*, 2013). Substrate mycelium will initiate development in sporulating cultures, and the compartments are separated by septa formed by membranes, then the mycelium cells undergo cell death while the viable cells are differentiated from a multinucleated mycelium by it has only sporadic septa, the aerial mycelium then grow from the surface hydrophobicity (Rioseras, 2014). The primary most important habitat for *Streptomyces* is soil, but also found in other places such as deserts, ice in the south pole, insects, plants, and sea (Cheng, 2015), depends on physical, chemical and biological factors such as food stress, temperature, pH, moisture, salinity, soil texture and climate.

These microorganisms are widely distributed in soil and makes 40% of soil bacteria. The dry and alkaline soil contain numerous populations of *Streptomyces* species and because the present of mycelium protect it from wind and rained eradication and gave the strength to soil texture (Hasani and Issazadeh, 2014).

Pathogenicity is certainly not a typical property of these organisms. Almost all of *Streptomyces* species are saprophytic, but a few are identified to be pathogens, potato scabs are important plant diseases caused by these species which appear as superficial or deep lesions of the tuber skin (Loria *et al.*, 2006). Two types of scabs cause separate diseases, common and netted scab, differing in symptoms produced. *Streptomyces scabiei* is the species isolated from common scab in potato growing countries and other *Streptomyces* species, including *Streptomyces acidiscabies* and *Streptomyces turgidiscabies* (Bouchek-Mechiche *et al.*, 2006).

The most interesting property of *Streptomyces* is the ability to produce secondary metabolites and enzymes including antibiotics, which important for *Streptomyces* species to kill other microorganisms that come in contact and play significant roles in drug discovery (Procopio *et al.*, 2012). Actinomycetes specially *Streptomyces* can produce a wide range of antibiotics. Almost of the worlds, known antibiotics are come from actinomycetes mostly from the genera *Streptomyces* and *Micromonospora* (Sheik *et al.*, 2017), about 80% of total antibiotics comes from *Streptomyces*; while the genus *Micromonospora* less than one-tenth as much as *Streptomyces* (Arifuzzaman *et al.*, 2010). Also, each *Streptomyces* species can produce 2-4 different bioactive molecules, the analysis of the genomic DNA reveals that each members of this genus encodes 20-40 biosynthetic pathways to the synthesis of potentially secondary metabolites (Egorov *et al.*, 2017). The genus *Streptomyces* provide a variety of new antibiotics importance in human health care and industrial application more than any other genus (Sajid *et al.*, 2011). The bioactive that associated with antibiotics from *Streptomyces* producers including antifungal, antibacterial, antiviral, antitumor and inhibition activities for enzymes (Chun *et al.*, 1997). Activation of antibiotic production by *Streptomyces* occurs by small molecules or environmental stresses; for example, phosphate limitations are required for the production of antibiotics, N-acetylglucosamine regulates the production of actinorhodin in *Streptomyces coelicolor*. Chemical factors such as goadsporin, hormaomycin and other synthetic compounds (Asamizu, 2015). *Streptomyces* are capable for resistance to a clinical antibiotic and are an important reservoir of antibiotic genes in soil (Kinkel and Schlatter, 2014). The gene clusters that encoded the biosynthesis of antibiotics also contain resistance genes for protect them from these compounds or modulate their signalling activity (Jiang, 2017).

The aim of this work is to study the characteristics of local *Streptomyces* stains isolated from soil and to assess antibiotic production and resistance of these isolates; moreover, attempts were carried out to ascertain the possible roles of plasmids in antibiotic production and resistance.

## MATERIALS AND METHODS

### Sample Collection

From January to July 2018, fifty clinical soil samples were collected from different regions in Al-Najaf Al-Ashraf province/Iraq. The soil samples were collected from 5-25 cm depth in sterile plastic container and transported to Microbiology Laboratory in Biology Department/Faculty of Sciences under ambient condition, then the samples pretreated by drying at 70°C in a hot air dryer for one hour.

**Table 1: This table showed biochemical tests for fifty samples isolated from local soil**

| Biochemical Tests | Place | Catalas Test | Oxidas Test | Urase Test | KIA Test | H2O production | MacConkey Agar Growth | Mannitol Salt Agar Growth |
|---|---|---|---|---|---|---|---|---|
| 1 | Al-Ghadeer | + | + | + | + | + | No Growth | No Growth |
| 2 | Kufa University | + | + | + | + | + | No Growth | No Growth |
| 3 | Al-Ameer | + | + | + | + | + | Growth | No Growth |
| 4 | Kufa University | + | + | + | + | + | No Growth | No Growth |
| 5 | A-Forat | + | + | - | + | + | Growth | No Growth |
| 6 | Al-Ameer | + | - | + | + | + | Growth | No Growth |
| 7 | Al-Ghadeer | + | + | + | + | + | No Growth | Growth |
| 8 | Al-Karama | - | + | + | + | - | Growth | No Growth |
| 9 | A-Forat | | + | + | + | + | No Growth | Growth |
| 10 | Al-Ghadeer | + | - | - | + | + | Growth | No Growth |
| 11 | Al-Ameer | - | + | + | + | + | Growth | No Growth |
| 12 | A-Forat | + | - | - | + | + | No Growth | No Growth |
| 13 | Al-Ameer | + | + | + | + | + | No Growth | No Growth |
| 14 | A-Forat | + | + | + | + | + | No Growth | No Growth |
| 15 | Al-Ameer | + | + | + | + | + | No Growth | No Growth |
| 16 | Al-North | - | - | - | + | - | No Growth | No Growth |
| 17 | Al-Ghadeer | + | + | + | + | + | No Growth | No Growth |
| 18 | Al-North | - | + | + | + | + | No Growth | No Growth |
| 19 | Al-Karama | - | - | + | + | + | Growth | No Growth |
| 20 | Al-North | + | + | + | + | + | No Growth | No Growth |
| 21 | Al-Karama | - | + | - | + | + | No Growth | Growth |
| 22 | Al-North | + | + | + | + | + | No Growth | No Growth |
| 23 | Kufa University | + | + | + | + | + | No Growth | No Growth |
| 24 | Al-North | + | + | + | + | + | No Growth | No Growth |
| 25 | Al-North | - | - | + | + | + | Growth | No Growth |
| 26 | Al-North | - | + | + | - | - | No Growth | Growth |
| 27 | Kufa University | + | + | + | + | + | No Growth | No Growth |
| 28 | Kufa University | + | + | + | + | + | No Growth | No Growth |
| 29 | Al-North | + | + | + | + | + | Growth | No Growth |
| 30 | Al-North | + | - | - | - | + | No Growth | No Growth |
| 31 | Desert Roads | + | + | + | + | + | No Growth | No Growth |

*Continued on next page*

*Table 1 continued*

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 32 | Al-Ghadeer | + | - | - | + | + | Growth | No Growth |
| 33 | Al-North | - | - | + | + | + | Growth | No Growth |
| 34 | Kufa University | + | + | + | + | + | No Growth | No Growth |
| 35 | Kufa University | + | + | + | + | + | No Growth | No Growth |
| 36 | Desert Roads | + | + | + | + | + | No Growth | No Growth |
| 37 | Desert Roads | + | + | + | + | + | No Growth | No Growth |
| 38 | Al-Karama | + | + | + | + | + | No Growth | No Growth |
| 39 | Al-Karama | + | + | + | + | + | No Growth | No Growth |
| 40 | Desert Roads | + | + | + | + | + | No Growth | No Growth |
| 41 | Desert Roads | + | + | + | + | + | No Growth | No Growth |
| 42 | Desert Roads | + | + | + | + | + | No Growth | No Growth |
| 43 | Kufa University | + | + | + | + | + | No Growth | No Growth |
| 44 | Desert Roads | + | + | + | + | + | No Growth | No Growth |
| 45 | Kufa University | - | - | - | + | + | Growth | No Growth |
| 46 | Desert Roads | + | + | + | + | + | No Growth | No Growth |
| 47 | Kufa University | + | + | + | + | + | No Growth | No Growth |
| 48 | Desert Roads | + | + | + | + | + | No Growth | No Growth |
| 49 | Desert Roads | + | + | + | + | + | No Growth | No Growth |
| 50 | Desert Roads | + | + | + | + | + | No Growth | No Growth |

The samples included five from Al-Ghadeer Quarter, five from Al-Karama Quarter, 10 from Kufa University, five from Al-Ameer Quarter, four from Al-Forat Quarter, 10 from North Quarters and eleven from desert roads in Al-Najaf.

**Isolation and Counting of *Streptomyces***

1g of each soil was added to 9ml of sterile distilled water and make serial dilution from $10^{-1}$ to $10^{-5}$, then 0.1ml of each diluted sample was placed onto the agar surface near one edge of the plates were prepared from Yeast extract Malt extract (YEME) agar medium. A flame sterilized wire loop was used to make five equally spaced streaks across the plate, then the plates incubated in the dark at 37°C for 7days. The colonies were counted as colony-forming unit per gram of soil sample, then the colonies that selected as presumptive *Streptomyces* colonies were subcultured on Tryptone Yeast extract (TYE) agar, then incubation at 37°C for 7 days.

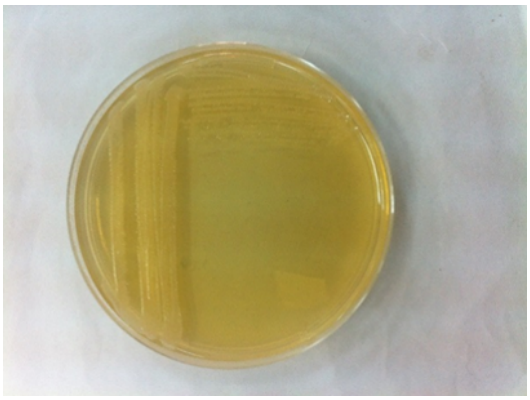

**Figure 1: This figure showed selective media (Yeast Extract MaltExtract Agar) for isolation of *Streptomyces* from soil**

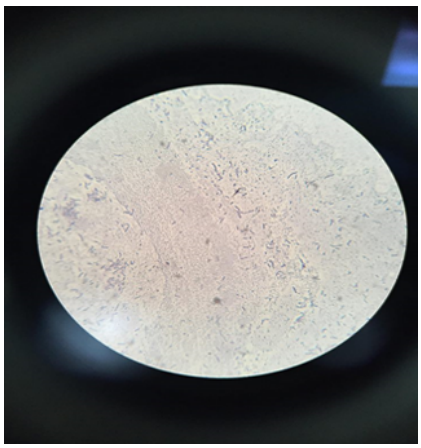

**Figure 2: Microscopic view for the isolate under 40x magnification showed gram positive with purple color**

**Biochemical Tests**

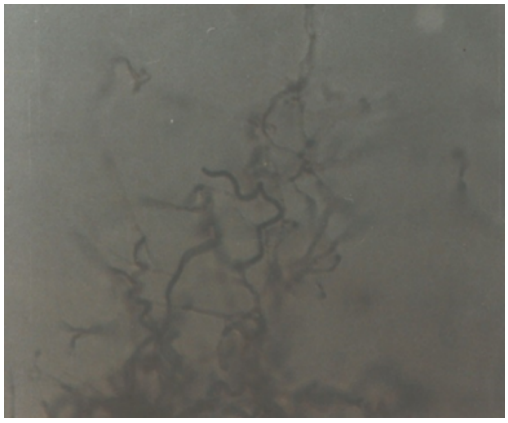

**Figure 3: Spiral shape of *Streptomyces* isolates under the digital microscope by 40x magnification**

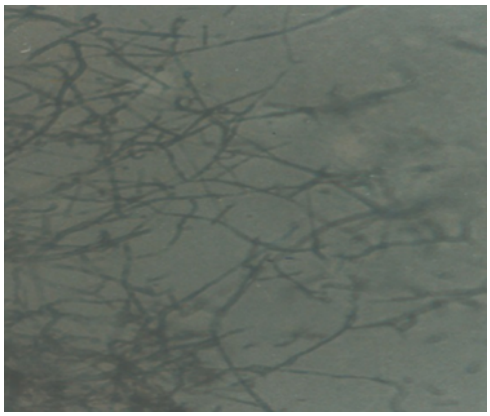

**Figure 4: This figure showed flexuous shape of *Streptomyces* isolates under the digital microscope by 40x magnification**

In this research we used seven biochemical tests for identification of *Streptomyces* isolates these are: Catalase test, Oxidase test, Urase test, Kligler Iron Agar test (KIA), Simmon's Citrate test (Hossain and Rahman, 2014) , addition to MacConkey agar test which inhibits gram-positive bacteria and Mannitol Salt agar which is selective media for *Staphylococcus* species.

**Preparation of Spore Suspension**

This method, according to (Hopwood, 1985).

**Preparation of Washed Inoculum**

This method, according to (Shirling and Gottlieb, 1966).

**Characteristics of Spore-Bearing Hyphae**

Determination of the characteristics of the spore-bearing hyphae and spore chains was done by direct microscopic examination of the culture surface on solid media. 40x resolution power were examined after 7 days of incubation.

**Antibiotic Sensitivity**

Five antibiotic discs were used for detection of antibiotic sensitivity of the *Streptomyces* isolates; these are Tetracycline (TE, 10mcg), Gentamycin (CN, 10mcg), Vancomycin (AV, 30mcg), Ampicillin (AM, 25mcg), Erythromycin (E, 10mcg). The culture medium used is Mueller Hinton Agar, 0.1ml of spore suspension were spreading by L-shaped spreader on this medium, then the antibiotic discs were seeded onto the agar surface. The plates were incubated at 37°C for 24hrs. The sensitivity of the antibiotics was observed by recorded the diameter of inhibition zone around the discs (Zothanpuia *et al.*, 2018).

**Determination of Antibacterial Activity**

The pasteur pipette was used to obtain agar plugs from each plate and then placed on plates of nutrient agar seeded with a lawn of test bacteria (*Staphylococcus aureus* and *E. coli*). The plates were stored at 4°C for 24hrs and then incubated at 37°C for 24hrs, then recorded the diameters of inhibition zone (Tememi, 1997).

**Isolation of Covalently Closed Circular DNA**

Plasmid isolation was done by alkaline lysis, according to (Kieser, 1984). This procedure is suitable for the rapid isolation of DNA from *Streptomyces*. The method consists of lysis of lysozyme by heated bacteria combined with alkaline denaturation of DNA at high temperature. Addition of a minimal amount of phenol/chloroform for renaturation of DNA and precipitation of single-stranded DNA together with protein. Then, phenol and further purification of the plasmid preparation is occurred by consecutive precipitations with isopropanol and spermine, followed by extraction with ethanol, producing samples suitable for restriction endonuclease digestion, ligation, and transformation.

**Detection of Plasmid DNA**

Agarose gel electrophoresis was done according to (Wood *et al.*, 1983).

**Curing of Plasmid DNA**

Acridine orange was used for plasmid DNA curing. Different concentrations of acridine orange were used (0,10,12,14,16,18,20,22,24 and 30) $\mu$g/ml in tryptone soya agar, these media poured in plates and inoculated with a proper dilution of spore suspension of *Streptomyces* isolates which gave 100-200 colonies per plate. The plates were incubated for 7 days at 37°C. after this period the growing colonies were counted to determine survival percentage, five colonies from a suitable plate which give 25-50% survival were examined for their antibiotic resistance on Mueller Hinton medium and on corn starch agar medium for antibacterial activity.

**RESULTS AND DISCUSSION**

From fifty soil samples, only thirty samples were grown on selective medium (Yeast extract Malt extract) agar (Figure 1). The colonies of *Streptomyces* were slow-growing, aerobic, glabrous or chalky, heaped, folded and produce aerial mycelium with long chains of spores and substrate mycelia of white colour; the colonies have an earthy odor.


**Figure 5: Showed oxidase positive at the left, Simmon citrate at the center, and KIA and $H2_O$ positive at the right**

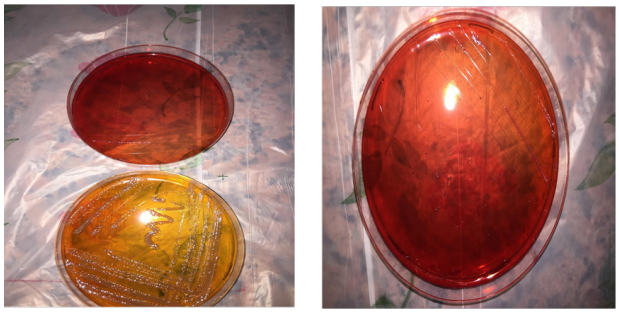


**Figure 6: A - Showed no growth of *Streptomyces* on MacConkey agar. While , B – the growth of *Proteus* spp.**

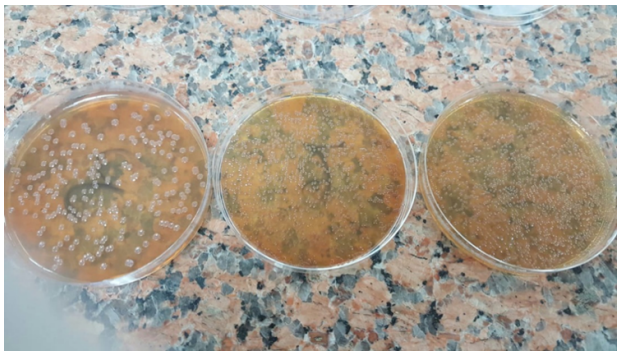

**Figure 7: Showed three petri dishes with three serial dilutions $10^{-1}$on the left, $10^{-2}$ in the center and $10^{-3}$ on the right by spread plate method**

The presumptive strains of *Streptomyces* were

**Table 2: The inhibition zone diameter to thirty isolates of *Streptomyces* against gentamycin and tetracycline antibiotics, while erythromycin, vancomycin and ampicillin no inhibition zones recorded**

| Antibiotic Discs | Gentamycin | Erythromycin | Tetracycline | Vancomycin | Ampicillin |
|---|---|---|---|---|---|
| 1 | 15 | - | 10 | - | - |
| 2 | 15 | - | 11 | - | - |
| 4 | 19 | - | 9 | - | - |
| 13 | 17 | - | 5 | - | - |
| 14 | 15 | - | 10 | - | - |
| 15 | 16 | - | 8 | - | - |
| 17 | 17 | - | 9 | - | - |
| 20 | 20 | - | 11 | - | - |
| 22 | 19 | - | 10 | - | - |
| 23 | 15 | - | 9 | - | - |
| 24 | 15 | - | 9 | - | - |
| 27 | 20 | - | 11 | - | - |
| 28 | 16 | - | 12 | - | - |
| 31 | 18 | - | 10 | - | - |
| 34 | 15 | - | 9 | - | - |
| 35 | 16 | - | 8 | - | - |
| 36 | 20 | - | 9 | - | - |
| 37 | 20 | - | 9 | - | - |
| 38 | 16 | - | 11 | - | - |
| 39 | 16 | - | 10 | - | - |
| 40 | 15 | - | 11 | - | - |
| 41 | 17 | - | 9 | - | - |
| 42 | 20 | - | 8 | - | - |
| 43 | 21 | - | 10 | - | - |
| 44 | 17 | - | 11 | - | - |
| 46 | 17 | - | 10 | - | - |
| 47 | 18 | - | 11 | - | - |
| 48 | 16 | - | 9 | - | - |
| 49 | 15 | - | 8 | - | - |
| 50 | 17 | - | 9 | - | - |

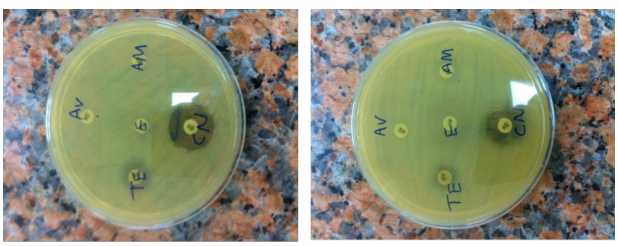


**Figure 8: Showed tow stains of *Streptomyces* tested against five antibiotics for revealed the antibiotic sensitivity**

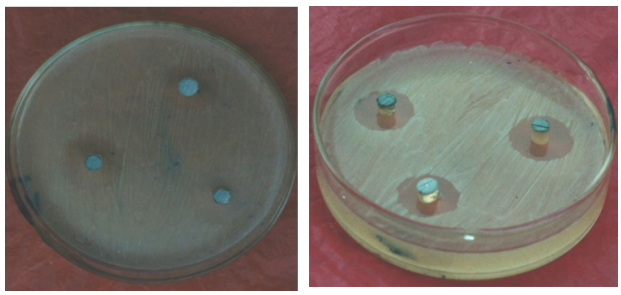

**Figure 9: Antibacterial activity for *Streptomyces* isolates against *S.aureus* at the left, while antibacterial activity against *E. coli* at the right**

stained by Gram-stain, all thirty isolates are Gram-positive (Figure 2).

Under the microscope, the morphology of spore

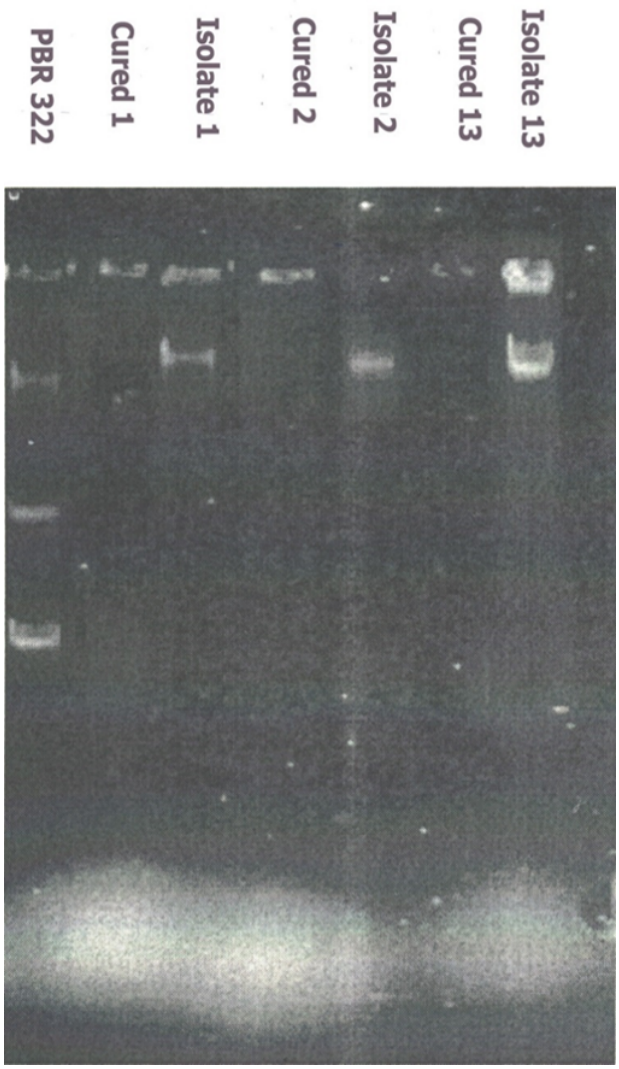


**Figure 10: Plasmid DNA bands of three isolates (1,2,13) and cured isolates (cured 1, cured 2, cured 13) without plasmid bands. PBR 322 : First-generation *E. coli* vector for DNA cloning. This plasmid has a low copy number (~20 copies per cell) due to the *rop* gene**

chains differs from species to others. The shape of spore chain of the isolates was divided into two groups: Spiral shape (Figure 3), the form was found ten isolates, and Flexuous shape (Figure 4) was found in twenty isolates.

The thirty isolates characterized by nonmotile, catalase-positive, oxidase-positive, urease positive and positive for kligler iron agar and H2S production (Figures 5 and 6), (Table 1). This table represented that the thirty *Streptomyces* strains were isolated from different regions included: eleven strain from desert roads, two from Al-Ghadeer Quarter, nine from Kufa University, two from Al-Ameer Quarter, one from Al-Forat Quarter, three from Al-North Quarter, and two from Al-Krama Quarter.

This result showed that the culture medium Yeast Extract Malt Extract agar was suitable and better from other medium Tryptone Yeast Extract agar for counting of *Streptomyces* after doing serial dilution ($10^{-1}$-$10^{-5}$) (Figure 7).

The results showed that the isolates were resistant to Erythromycin, Tetracycline, Vancomycin, and Ampicillin, but on the other hand, these isolates were sensitive to Gentamycin (Figure 8), (Table 2).

The results showed that Tryptone Yeast Extract agar was a suitable medium for detection of antibacterial activity of thirty isolates used in this study, while Yeast Extract Malt Extract agar was found to be not suitable for detection of antibacterial activity of the isolates. Three isolates (17, 22, 37) showed maximum inhibition zone against *E. coli* (15mm) and *S. aureus* (13mm), (Figure 9).

Lower molecular weight CCC-DNA were detected from all strains. There was a similarity between the strains in their profile. The strains exhibited one plasmid (Figure 10).

Three isolates were selected for curing experiments these are: 1, 2, 13. The results showed that *Streptomyces* viability was affected with the increase in acridine orange concentration. The percentages of grown colonies at the following concentrations: 16,18,20,22, and 24 $\mu$g/ml of acridine orange were 41%, 29%, 20%, 16% and 12% for the isolate 1, whereas for the isolate 2 were 57%, 35%, 25%, 17%, and 10%, while the percentages of grown colonies for the isolate 13 were 55%, 40%, 30%,21% and 17% respectively. 20$\mu$g/ml of acridine orange was selected in this study for curing experiments, the growing colonies after acridine orange treatment were checked for tetracycline resistance, the selected cured colonies which were tetracycline sensitive and checked further for their antibiotics and antibacterial activity.

The result showed that the obtained cured tetracycline sensitive colonies were also sensitive to erythromycin, vancomycin, and ampicillin. Also, the cured colonies had lost their antibacterial activity. The lost of antibacterial activity, and antibiotic resistance might indicate that the plasmid was involved in antibiotic production and resistance.

Soil is a treasure for a large number of organisms living together, some of them are harmful, causing diseases for plants, animals and human beings. Other organisms are useful by producing beneficial natural products, including antibiotics.

In my study, we hard work tried to isolate, identify and characterize active strains of Streptomyces with tests of antibiotic production and antibacterial activ-

ity against gram-positive and gram-negative bacteria from numerous samples of soil collected from different places in Al-Najaf Al-Ashraf provinces.

Depending of a large number of actinomycetes in soil, 99% of species has still been unknown. Isolation of species from natural habitats increases the probability of discovery of new types of antibiotics (Davies and Procópio, 1999).

The growth of *Streptomyces* on agar media is very important because it's needed for isolation, identification and production of secondary metabolites and various genetic manipulation protocols such as electroporation and conjugal transfer of DNA (Shepherd, 2010).

In this study was counted the number of colonies on each medium (YEME agar and TYE agar) and represented the YEME agar medium was the best media for isolation of Streptomyces from the soil compared to other media, this study agreed with the study of (Wadetwar and Patil, 2013).

The increased resistant to antibiotics by microorganisms makes the treatment of infectious diseases are very difficult. Therefore, the development of novel drugs against the pathogenic microorganisms is needed; recently, the researchers showed the potential of habitats as reservoirs of antimicrobial compounds producers (Mohamed, 2017).

From thirty isolates of *Streptomyces*, 20 samples were inhibited gram-positive bacteria, 5 samples were inhibited gram-negative bacteria, and 5 had activity against both groups. These results agreed with other studies that showed almost *Streptomyces* strains isolated from soil had activity against gram-positive bacteria (Thakur *et al.*, 2007).

The variation in antibacterial activity on different kinds of culture media might be due to the composition of these media. High production of antibiotics has been achieved by cultivating the producing organisms in media containing slowly utilized carbon and nitrogen sources or under conditions which allow a slow supply of these nutrients (Drew and Demain, 1977).

On the other hand, the suitable temperature for the detection of antibacterial activity was 37°C, under this temperature the isolates showed antibacterial activities and kill test bacteria, while 45°C was not suitable for detection of antibacterial activity, under this temperature no inhibition zone was observed around any isolate this due to the effect of high temperature on the activity of the transport systems that responsible for antibiotic production, and other important physiological and biochemical function of the microbial cell (Egorov *et al.*, 2017).

The absence of plasmid DNA in the cured colonies was determined by (Kieser, 1984) method. The Figure 10 revealed that the suspected plasmid cured colonies lost their plasmids as compared with mother strains that have a single band of plasmid DNA at the same distance. This result was an agreement with some studies of actinomycetes isolates such as (Omura *et al.*, 1981) they showed exist one plasmid in *Streptomycesambofacien* KA-1028, *Streptomyces hygroscopicus* KCCS-0439 and *Streptomyces cirratus* JTB-3. While, other studies showed that some *Streptomyces* species containing two or more plasmids reach in some of them to four (Czernik, 1983).

The genus of *Streptomyces* was carried extrachromosomal elements (plasmids) are present in the form of Covalently Closed Circular (CCC) DNA, sometimes are present as linear elements. *Streptomyces* plasmids were carried genes encoded protein responsible for the genetic control of the biosynthesis of antibiotic (Nithya, 2008). Although these bacteria contain antibiotic biosynthetic gene clusters, they also had specific resistance genes for protecting themselves from their own compounds. Since a *Streptomyces* strain contains about 20 different gene clusters for the production of antibiotics, they form an enormous pool of antibiotic resistance genes in the soil, which can be passed to another bacteria by horizontal gene transfer (Muth, 2012). The genes of *Streptomyces* involved in secondary metabolite production be located as clusters on the chromosome, also, several studies have recognized biosynthetic clusters on the linear plasmids that produce important secondary metabolites (Cornell *et al.*, 2018).

## CONCLUSION

We conclude in this study only thirty isolates from fifty soil samples were grown on selective medium and positive for biochemical tests, all isolates were resistance to Erythromycin, Tetracycline, Vancomycin, and Ampicillin but on the other hand, these isolates were sensitive to Gentamycin. The isolates had one plasmid at the same distance responsible in antibiotic production and resistance; these antibiotics were acting against gram-positive bacteria such as *Staphylococcus aureus* and gram-negative bacteria such as *E. coli*.

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