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

Suad A Al-Hilu*1, Wisam H Al-Shujairi2

1Department of Biology, Faculty of Sciences, University of Kufa, Iraq
2Department of Clinical Laboratory Sciences, Faculty of Pharmacy, University of Babylon, Iraq

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, ten from Kufa University, five from Al-Ameer Quarter, four from Al-Forat Quarter, ten 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.

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-
Streptomyces, the other one 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 nitrates, 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 and , 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. (Boucek-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, 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 (Gouse 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 and Esnault, 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.

© International Journal of Research in Pharmaceutical Sciences 2143
Table 1: This table showed biochemical tests for fifty samples isolated from local soil.

| Biochemical Tests | Place            | Catalase Test | Oxidase 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
|   | Location               | Growth | No Growth |
|---|------------------------|--------|-----------|
| 32| Al-Ghadeer             |        |           |
| 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| Kufa University        | 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 7 days. 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.

**Biochemical Tests.** 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, 25mcg).
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) μg/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 H2O 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 stained by Gram-stain, all thirty isolates are Gram-positive (Figure 2).

Under the microscope, the morphology of spore chains differs from species to others. The shape
Table 2: showed 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 of spore chain of the isolates was divided into two groups: Spiral shape (Figure 3), the form was found

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

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 μg/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. 20μg/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 activity 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 (YME agar and TYE agar) and represented the YME agar medium was the best media for isolation of Streptomyces from the soil compared to other media, this study agreed with the study of (Pati, 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 , 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 and Esnault, 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 Streptomyces ambofacien 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).

CONCLUSIONS

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.

ACKNOWLEDGEMENT

I would like to present my great thanks and appreciation to the staff of Microbiology Laboratory in the Faculty of Sciences/ University of Kufa/ Iraq for their helpful guidance and great efforts that made this work possible.
REFERENCES

Al-Saadi, A., N. M. H. 2013. Eman Mohammad Jaralla. Isolation and Identification of Streptomyces from Different Sample of Soils. Journal of Biology and Medical Sciences - JBMS, 1:31–36.

Arifuzzaman, M., Khatun, M. R., Rahman, H. 2010. Isolation and screening of actinomycetes from sundarban soil for antibacterial activity. Afr. J. Biotechnol, 9:4615–4619.

Asamizu, S. 2015. Killing of Mycolic Acid-Containing Bacteria Arrested Induction of Antibiotic Production by Streptomyces in Combined-Culture. PloS one, 10(11):142372–142372.

Bouchek-Mechiche, K., L. G., Andrivon, D., Normand, P. 2006. Streptomyces turgidiscabies and Streptomyces reticuliscabiei: one genomic species, two pathogenic groups. International Journal of Systematic and Evolutionary Microbiology, 56:2771–2776.

Cheng, K. 2015. Population genetic analysis of Streptomyces albidovalavus reveals habitat barriers to homologous recombination in the diversification of streptomyces. Applied and environmental microbiology, 81(3):966–975.

Chun, K., Hah, M. Y., Kang, Y. C., S. O. 1997. Streptomyces seolensis sp. Novel. Int. J. Syst Bacterial, 47:492–498.

Cornell, C. R., Marasini, D., Fakhr, M. K. 2018. Molecular Characterization of Plasmids Isolated From The Great Salt Plains of Oklahoma Using PFGE and Next Generation Whole Genome Sequencing. Frontiers in microbiology, 9.

Czernik, P. 1983. Isolation and characterization of Streptomyces erythreus plasmids. Acta microbiologica Polonica, 32(4):327–337.

Davies, J., Procópio, R. E. D. L. 1999. Antibiotics produced by Streptomyces. The Brazilian Journal of Infectious Diseases, 15(12):466–471. Trends in Genetics.

Drew, S. W. A. D., A. L. 1977. Effect of primary metabolites on secondary metabolism. Ann. Rev, 31:343–356.

Egorov, N. S., Esnault, C. A. . 2017. Strong antibiotic production is correlated with highly active oxidative metabolism in Streptomyces coelicolor M145. Scientific Reports, 7(1):200–200.

Gouse, Sheik, M., Maqbul, G. S., S. R. S., Ms. Isolation, Characterization, Actinomycetes, Soil, Ad-Dawadmi, S., Arabia, Antibacterial, S. T. Activities 2017. International Journal of Pharmacy and Phamaceutical Sciences, 9(10):276–279.

Hasani, A. K. A., Issazadeh, K. 2014. Streptomyces: Characteristics and Their Antimicrobial Activities. International journal of Advanced Biological and Biomedical Research, 2(1):63–75.

Hopwood, D. A. 1985. Production of 'hybrid' antibiotics by genetic engineering. Nature, 314(6012):642–644.

Hossain, M. N., Rahman, M. M. 2014.

Jiang, X. 2017. Dissemination of antibiotic resistance genes from antibiotic producers to pathogens. Nature Communications, 8:15784–15784.

Kieser, T. 1984. Factors affecting the isolation of CCC DNA from Streptomyces lividans and Escherichia coli. Plasmid, 12(1):19–36.

Kinkel, L. L., Schlatter, D. C. 2014. Global biogeography of Streptomyces antibiotic inhibition, resistance, and resource use. FEMS Microbiology Ecology, 88(2):386–397.

Loria, R., Kers, J., Joshi, M. 2006. Evolution of plant pathogenicity in Streptomyces. Annual review of phytopathology, 44:469–487.

Mohamed, H. 2017. Isolation and Characterization of Actinobacteria from Algerian Sahara Soils with Antimicrobial Activities. International journal of molecular and cellular medicine, 6(2):109–120.

Muth, L. T. G. 2012. Conjugative DNA transfer in Streptomyces by TraB: is one protein enough? FEMS Microbiol Lett, 337:81–88.

Nithya, P. P. 2008. Plasmid DNA of Antibiotic Producing Strains of Streptomyces sannanensis Isolated from Different States in Southern India. Biotechnology, 7:487–492.

Omura, S., Ikeda, H., Tanaka, H. 1981. Extraction and characterization of plasmids from macrolide antibiotic-producing streptomycetes. The Journal of antibiotics, 34(4):478–482.

Patil, R. W. A. A. T. 2013. Isolation Characterization Bioactive From Nagpur. INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH, 24:1428–1433.

Procopio, R. E. 2012. Antibiotics produced by Streptomyces. The Brazilian journal of infectious diseases: an official publication of the Brazilian Society of Infectious Diseases, 16(5):466–471.

Rioseras, B. 2014. Mycelium differentiation and development of Streptomyces coelicolor in lab-scale bioreactors: programmed cell death, differentiation, and lysis are closely linked to undecyldipropargylamin and actinorhodin production. Bioresource technology, 151:191–198.

Sajid, I., Shaaban, K. A., Hasnain, S. 2011.

Shepherd, M. D. 2010. Laboratory maintenance of
Streptomyces species. Current protocols in microbiology, 10. Chapter.

Shirling, E. B., Gottlieb, D. 1966. Methods for characterization of Streptomyces species1. International Journal of Systematic and Evolutionary Microbiology, 16(3):313–340.

Smaoui, S. A. M., Florence, F., Fguira, L. B., , M., G., . M. 2011. Lofti Taxonomy and antimicrobial activities of a new Streptomyces sp. TN17 isolated in the soil from an oasis in Tunis. Archives of Biological Sciences, 63(4):1047–1056.

Taddei, A. 2006. Isolation and identification of Streptomyces spp. from Venezuelan soils: morphological and biochemical studies. I. Microbiological research, 161(3):222–231.

Tememi, M. A. 1997.

Thakur, D., Gogoi, Y. A., Bora, B. K., T. C. 2007. Isolation and screening of Streptomyces in soil of protected forest areas from the states of Assam and Tripura, India, for antimicrobial metabolites. J. Med. Mycol, 17:242–249.

Wood, E. J., Fritsch, J. E. F., Sambrook 1983. Molecular cloning, A laboratory manual by T Maniatis. Biochemical Education, 11(2):82–82.

Zothanpuia, A. K. P., Yadav, B. P. M. K., Singh 2018. In vitro evaluation of antimicrobial activities and antibiotic susceptibility profiling of culturable actinobacteria from fresh water streams. Indian Journal of Experimental Biology, 56:665–673.