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Evaluation of the possibility of direct isolation *Streptomyces* DNA from soils using Real Time PCR and compared to Conventional PCR

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A B S T R A C T

When studying bacterial species, the traditional methods of isolation and laboratory purification were essentially used that required effort and time, which necessitated searching to discover modern technologies to save time and effort to reach same goal. In our study real time was used for amplification Direct DNA isolation chain reaction from different types of soil in Nineveh Governorate, 50 samples were collected, 8 of them were selected to study and apply a comparison between the traditional isolation of *Streptomyces* DNA and the direct isolation of DNA from the soil using PCR and the real-time amplification chain reaction. The first traditional method of DNA isolation showed *Streptomyces* presence in selected soil samples. The result was confirmed by using16sRNA, as a second method, direct DNA extraction of eight different soil samples was carried out using PCR and real-time PCR. The PCR result on the electrophoresis gel showed *Streptomyces* presence in 1 of 8 samples, and by performing the Real-time PCR (qPCR) using second method, two samples from eight isolates showed their negativity i.e. no presence of *Streptomyces* in it, maybe the soil samples not contain these bacteria or increase concentration of DNA. The specific primer of *Streptomyces* made it difficult to bind to the DNA pieces. Six samples showed the positive result of qPCR and proved *Streptomyces* presence in it, this result shedding lights the use of qPCR in the diagnosis of DNA isolated directly to the sample and provision the effort and time spent by diagnosing by the traditional method, and also ensures the possibility of relying on the qPCR as one of the sensitive and accurate methods in the diagnosis and identification of species.

تقييم إمكانية العزل المباشر للحمض النووي *Streptomyces* من عدة ترب باستخدام تفاعل البوليميريز المتسلسل في الوقت الحقيقي ومقارنته بتفاعل البوليميريز المتسلسل التقليدي

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الخلاصة:

عند دراسة الأنواع البكتيرية، تم استخدام الطرق التقليدية للعزل والتنقية المختبرية بشكل أساسي والتي تتطلب الجهد والوقت، مما استلزم البحث لاكتشاف التقنيات الحديثة لتوفير الوقت والجهد للوصول إلى نفس الهدف. لذلك في دراستنا، من أنواع مختلفة من الترب تم جمع 50 عينة، وتم اختيار 8 منها لدراسة وتطبيق مقارنة بين العزل التقليدي للحمض النووي*Streptomyces* والعزل المباشر للحمض النووي من التربة باستخدام تفاعل البوليميريز المتسلسل وتفاعل التضخيم المتسلسل في الوقت الفعلي. أظهرت الطريقة التقليدية الأولى لعزل الحمض النووي وجود *Streptomyces* في عينات التربة المختارة. تم تأكيد النتيجة بواسطة تفاعل البوليميريز المتسلسل، ثم تم إجراء الطريقة الثانية عن طريق الاستخراج المباشر للحمض النووي. أظهرت النتيجة على جل الرحلان الكهربائي وجود *Streptomyces* في 1 من 8 عينات، ومن خلال إجراء تفاعل البوليميريز المتسلسل في الوقت الفعلي باستخدام الطريقة الثانية، أظهرت عينتان من ثماني عزلات سلبيتهما، أي عدم وجود *Streptomyces* فيه، ربما لا تحتوي عينات التربة على هذه البكتيريا أو زيادة تركيز الحمض النووي. جعل البرايمر الخاص بـ *Streptomyces* من الصعب الارتباط بقطع الحمض النووي. أظهرت ست عينات النتيجة الإيجابية لتفاعل التضخيم المتسلسل في الوقت الفعلي وأثبتت وجود *Streptomyces* فيه، مما يسلط الضوء على استخدام الطريقة المباشرة لاستخراج الحمض النووي وتقصير الجهد والوقت في دراسة المجتمعات الميكروبية بشكل عام وخاصة *Streptomyces* في التربة، كما تضمن إمكانية الاعتماد على تفاعل البوليميريز المتسلسل في الوقت الفعلي كأحد الطرق الحساسة والدقيقة في تشخيص وتحديد الأنواع.

الكلمات الرئيسية: الاستخلاص المباشر DNA، تفاعل البوليميريز المتسلسل التقليدي، تفاعل البوليميريز المتسلسل الكمي، Streptomyces، srRNA16.

1-INTRODUCTION

The genus *Streptomyces* represents 80% of the total number of Actinomycetes populations, which are an important major component of the soil microbial populations [1]. Aerobic bacteria are considered one of the few species, such as *Pseudomonas aeruginosa*, that can rule the microbial community, as it spreads in various soils and different environments, even in soils and the environment that are nutritionally poor for the high ability of these bacteria to adapt to various environmental conditions [2].

It produces a wide spectrum of bioactive secondary metabolic substances, as it crosses the main source of antibiotics, which constitute 80% of the total antibodies produced by Actinomycetes, where it produces bioactive metabolites with broad-spectrum activities of antibacterial, antiviral, fungi, parasites and

antitumor agents [3]. These bacteria form multinucleate filaments, which are an important characteristic of prokaryotic organisms, they form vegetative mycelium embedded in a nutrient medium from which vertical branches branch (aerobic mycelium), which later forms a series of spores [4,5].

With the increase in scientific studies, the reliance on molecular biological techniques instead of traditional cultivation techniques in the census and detection of microbial groups has increased, as culture media are used that may be selective for some bacterial species without other species, even if many food media are used, many species will not grow in the laboratory, as research indicates that the percentage of what grows from bacterial communities on laboratory food media does not exceed 1% of the bacteria present in soil environment [6,7,8], so alternative molecular biological techniques should be used in microbial community analysis and species study. Soil is the largest reservoir for diversity, microbial communities, microorganisms, and other organisms, 1 gram of which contains 100,000-100,000,000 of various bacterial species [9].

Fungi and bacteria are a large part of this ratio, but one of the most difficult challenges is to develop procedures for isolating and purifying certain species, especially because the soil contains an overlapping and complex matrix of humic and organic materials that prevent or hinder the work of restriction enzymes or interfere with their mechanism of action, especially with a high content of organic matter, where complex mixtures of compounds show wide aggregates of solubility that make the separation process difficult, if not impossible [10].

Research has been conducted on extraction and separation conditions through which all humic and organic substances can be removed from soil DNA extract, and several methods of DNA purification have been used, but none of these methods has proven to be strong and general for acceptance within practical techniques as a basic protocol. Extracting DNA from the soil directly does not require any cultivation on food media in the laboratory or the development of bacteria, as the protocol of action can be divided into two groups according to the mechanism used to break up soil particles and remove bacteria adhering to them, which includes either the use of physical methods or chemical methods [11].

Each of these methods has negative and positive aspects in terms of their efficiency in extracting DNA with good purity, as most direct extraction protocols begin with the steps of breaking cells by physical or chemical methods such as bead beating cycles, freezing or thawing followed by the addition of detergents such as Sodium Dodecyl Sulfate (SDS) in addition to heat treatment with a buffer solution such as Tris-HCl or sodium phosphate buffers, in addition to use of

chelating agents such as Ethylenediaminetetraacetic acid (EDTA) to protect DNA from its digestive enzymes abundant in the external environment or through enzymatic digestion with lysozyme or protease and then extracting organic matter and sedimentation of alcohol and usually needs further purification to remove inhibitory substances from the extract, the methods of extracting DNA directly from the soil are not an easy method in all cases as they are complicated due to humic substances that inhibit the action of PCR and its enzymes, as many attempts have been made in direct extraction of DNA, but most of them have not been able to achieve the desired goal enough to be generally accepted as a standard [12].

2-MATERIALS AND METHODS

2-1- Sample Collection

Collecting 50 samples from different areas of different soils in Nineveh Governorate, including agricultural lands, forest areas, rocky soils, public areas and public parks, and from some areas of oil fields, and soil sample types included sand, clay, sand-clay, rocky and some river edge areas, where samples were taken randomly using a special soil shovel from these areas, as approximately 100 grams of each soil sample with a depth of about 10-15 cm were taken after removing about 3 cm from the soil surface to avoid contaminated surfaces, these samples were placed in sterile plastic cans that were sealed after the samples were collected tightly and immediately transported to the laboratory. Some physicochemical properties of the soil, including texture, color, pH, and moisture content, have been determined [13], later it was dried at 70°C for one hour in a hot air oven .

2-2- The Traditional Method of DNA Isolation

2-2-1-Isolation of *Streptomyces*

After pre-drying, *Streptomyces* were isolated by spreading the samples by sterile swab on International Streptomyces Project(ISP) media, which contains large amounts of nutrients, including peptides, vitamins, nutrients, and minerals, where one gram of soil samples was suspended in 9 ml of sterile distilled water. After shaking and stirring to obtain homogeneity, a series of dilutions were made up to 10^{-5} and three repeats done. Then, take 100 μl of each dilution by micropipette and put it in the dish and then pour the medium in a petri dish with stirring the medium (that was added to it 25 µg/ml of ampicillin and 50 µg/ml of Nystatin to restrict the growth of gram-negative and fungal contaminants) to distribute and homogenize the samples with the medium respectively, and incubate the dishes at 28°C for 7-14 days. Individual non-overlapping colonies

were then selected that appeared similar to *Streptomyces* colonies and then cultivated several times to obtain a pure colony [13,14].

2-2-2-*Streptomyces* purification

After incubation of dishes, the presence of *Streptomyces* colonies was observed, which are often in the form of cretaceous colonies or viscous property with dry wrinkles and which appear under optical microscopy branched filaments with or without aerobic mycelia[15].*Streptomyces* colonies were replanted on the medium of agar yeast extract – malt extract agar (ISP2) and incubated at 28 °C for 7-14 days to obtain pure isolates and colonies were maintained individually on culture media. After maintained in glycerol stocks at 25% (v/v) before being stored at –80°C until used.

2-2-3-Morphological and Biochemical Characterization Analyses of *Streptomyces* Isolates

Microscopically, 8 pure isolates were tested as all *Streptomyces* were distinguished based on the filamentous shape of cells, morphology and forms of spores, terrestrial vegetative mycelium color, and aerobic mycelium color, and Gram stain was performed to facilitate microscopic characterization, isolate growth was distinguished using different media such as International *Streptomyces* Project media (ISP2) and tryptone-soy agar, where the biochemical properties of *Streptomyces* isolates have been identified and evaluated as described [16].

2-2-4-Preparation of Bacterial Broth Culture

To prepare the nucleic acid extraction medium, 50 ml of ISP2 medium supplemented with 50 μg/ml of ampicillin was inoculated in a sterile glass vial of 100 ml with a loop campaign of purified isolates and then all bottles were incubated at a temperature of 28 °C with a vibrating incubator at a speed of 151 cycles per minute for 3-4 days for good isolates growth [17].

2-2-5-DNA Extraction and PCR-amplification

The genomic DNA was extracted for each isolation separately, where the liquid nutrient medium was transferred to special tubes of the centrifuge to separate the bacterial cells from the nutrient medium at a speed of 13,000 cycles per minute for 10 minutes. Then, the precipitate was washed with 2 ml of Phosphate Buffered Saline (PBS) to purify it and then subjected it to DNA extraction. According to the Geneaid protocol, the precipitate took the equivalent of 1.5 ml in the Eppendorf tubes and then added 200 μL of the enzyme Lysozyme at a concentration of 0.8 mg/ml and then mixed with a vortex. Then the enzyme

proteinase K was added and mixed, after that put the tubes in a water bath at 60°C for 10 minutes with stirring every three minutes then 200 µL of GB solution was added and placed in a water bath at a temperature of 70°C with stirring every three minutes. Then add 200 µL absolute ethanol with manual mixing and then transfer the mixture to a GD tube that was centrifuged at a speed of 16000 for 2 minutes. Neglect the precipitate in a column tube. After that, 400 µL of the first washing solution was added to GD tube, then to the centrifuge, and then 600 µL of the second washing solution was added and centrifuged. The steps ended with the addition of elution and after the end of the extraction process, the purity of the DNA was measured with the Qubit 4 device, then the amplification chain reaction of the samples is performed with the normal PCR reaction .

2-3-The Direct Method of DNA Isolation

Based on the protocol for Geneaid for direct soil extraction, 300 mg of soil sample was taken and placed in a special bead-beating tube, 750 µL of SL1 buffer is added to it and mixed well, then 150 µL of SL2 buffer is added to the bead-beating tube. Then mixed for 5 seconds, then placed at a temperature of -4°C for 5 minutes, then a centrifuge is performed at a speed of 8000 cycles/minute at room temperature to precipitate the unreactive molecules. and PCR inhibitors, then 500 µL is transferred from the clear filtration to the Inhibitor Removal Column placed in 2 ml centrifuge tubes and placed in the centrifuge at 16,000 cycles for a minute at room temperature and then keeps the precipitate in a 2 ml centrifuge tube. 900 µL of SL3 Buffer is added and 750 µL is transferred to the GD Column placed inside 2 ml Collection Tube and centrifuged at 16000 cycles/minute at room temperature and then GD column is transferred to a new 2 ml Collection tube, 400 µL of SL3 Buffer is added to the centrifuge and then 600 µL is added. From the wash buffer solution, centrifuged twice, then elution is added, and after the extraction process, the purity of the DNA is measured with a Qubit 4 device, and then a chain amplification reaction is performed for the samples with the usual PCR reaction.

2-3-1-Polymerase Chain Reaction (PCR)

16S rRNA gene specific of *Streptomyces* amplification reaction was performed, adding 5 µL of DNA from each sample to a mixture consisting of 12.5 µL master mix, 1 µL from each forward primer and reverse primer, and 5.5 µL free nuclease water per tube, and after mixing well. The following heat cycle was performed: 94 °C 3 min (one cycle), 94 °C 30 sec, 53 °C for 45 sec, 68 °C 30 sec (35 cycles), 68 °C 7 minutes (one cycle).

2-3-2-Real Time Polymerase Chain Reaction

The mixture is prepared in a size of 20 µL including 10 µL master mix, 0.5 µL of each forward primer and reverse primer, 5 µL of DNA, and 4 free nuclease waters for each sample. The material is placed in a 0.2 ml PCR tube and placed for 10 seconds in the centrifuge until the components on the walls of the inner PCR tube are deposited. The tubes were then inserted into the quantum polymer chain reaction device carefully to complete replication with cycles that included 95°C for 8 min (1 cycle), 95°C for 15 sec, 60°C for 30 sec (50 cycles), 60-96°C for 20 min (1 cycle).

2-3- 3-Gel Electrophoretic Analysis of PCR Product

A small fraction (4 µL) of each PCR solution was analyzed in the gel pits of 1% w/v agarose gel molded and operated in TBE insulator (pH 8.3), the Red Safe gel was stained, after using a special ladder, the power supply voltage of 110V was stabilized for 65 minutes, and imaged using UV light [18].

3-RESULTS AND DISCUSSION

3-1-Sample collection, bacterial isolation and purification

From different and varied soils, 50 samples were collected, including clay, sandy, and silt soils from agricultural lands and from loamy soils for shallow river edge areas, which contain high percentages of carbon sources and organic matter, which encourages microbial biomass in increasing the functional diversity of food sources in the soil [19].

ISP Medium supported with the antibiotics Nystatin and Ampicillin were used to inhibit the growth of fungi and a wide range of other bacterial species [20], and after laboratory transplantation, pure agricultural media technique, microscopic examination and biochemical tests were performed to confirm them. The tests showed the presence of bacteria in 37 samples of different soil, which enhances the ability of bacteria to adapt to the diversity of different environments [21]. All isolates observed were filamentous bacilli, Gram-positive, and negative acid-fast stain[22] (Figure 1).

The colonies were grown with a period of 5-7 days to support the slow gradual growth of the colonies, which were identified from the beginning by their shapes, including chalky, hard, and leathery colonies that attach to the surfaces of agar in the form of a vegetative mycelium, whose peaks end with a series of spores, and the colonies also showed different colors, including brown, gray and orange due to their ability to produce melanin, especially on their own media, ISP, in addition to their distinctive smell, which resembles the smell of dirt [23,24]. Four of these well-purified isolates were selected for study .

3-2-Phenotypic Properties

Streptomyces can be distinguished by their ability to produce bacterial filaments consisting of two types of hyphae: aerial hyphae and vegetative hyphae that are instilled in the nutrient medium, from which the aerobic mycelium later formed creates a series of spores on their apical edges, and these parts have the ability to produce a wide range of pigments that may be different among themselves, as this ability was used to determine the *Streptomyces* [25].

a

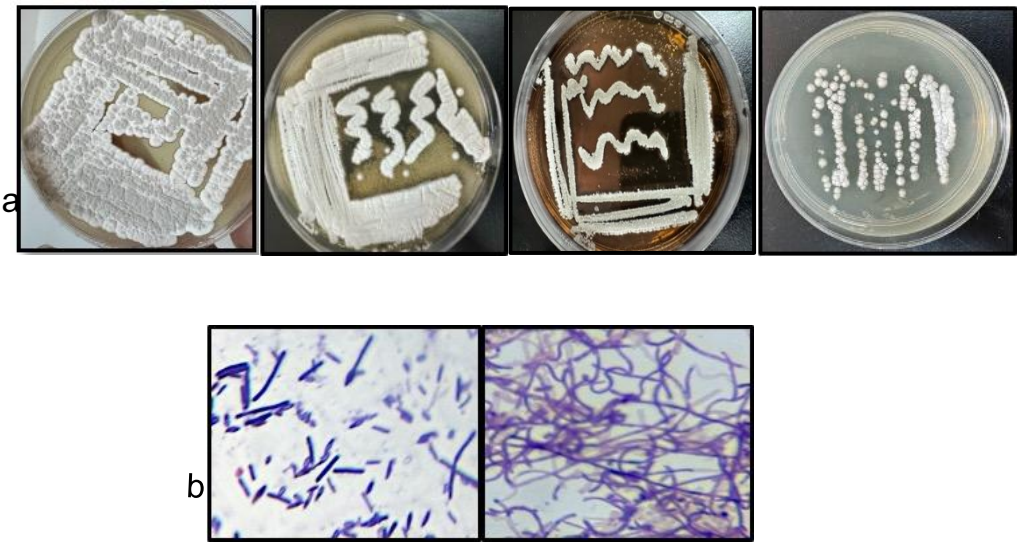

Figure (1): (a) Colony morphology and Color of *Streptomyces* sp. grown on ISP; (b) microscopic view of *Streptomyces* sp. Mycelium morphology

3-3-Molecular Properties

Streptomyces sp.CU16-1 gene is a partial sequence from the 16srRNA site was amplified for selected isolates of 1411 bp size by both primers, pA(forward) and pH (Revers) designed by [26], and used by [27,28], as described by [29]. were used as the polymerase chain reaction amplified for one cycle of the initial denaturation phase at 94°C for 3 minutes and then 35 cycles of 94°C for 30 seconds, the annealing phase at 54°C for 30 seconds, the extension at 70°C for 30 seconds, and a final 7-minute extension step at 70°C. All isolates showed a positive result confirming the chemical diagnosis of the selected isolates.

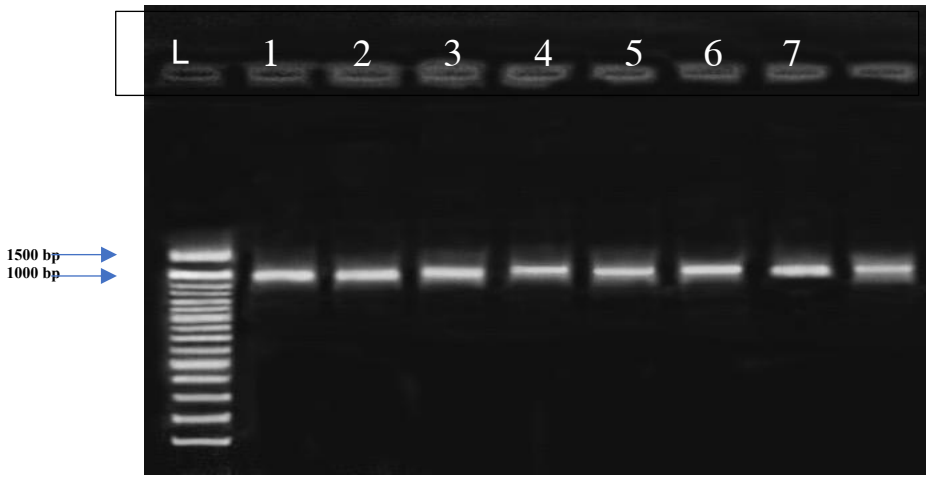

Figure (2): Agarose gel electrophoresis of PCR amplification products of genomic DNA isolated from *streptomyces* pure culture: lane L, DNA ladder; lanes 1, 2, 3, 4, 5, 6, 7, and 8 showed different isolates of *Streptomyces*

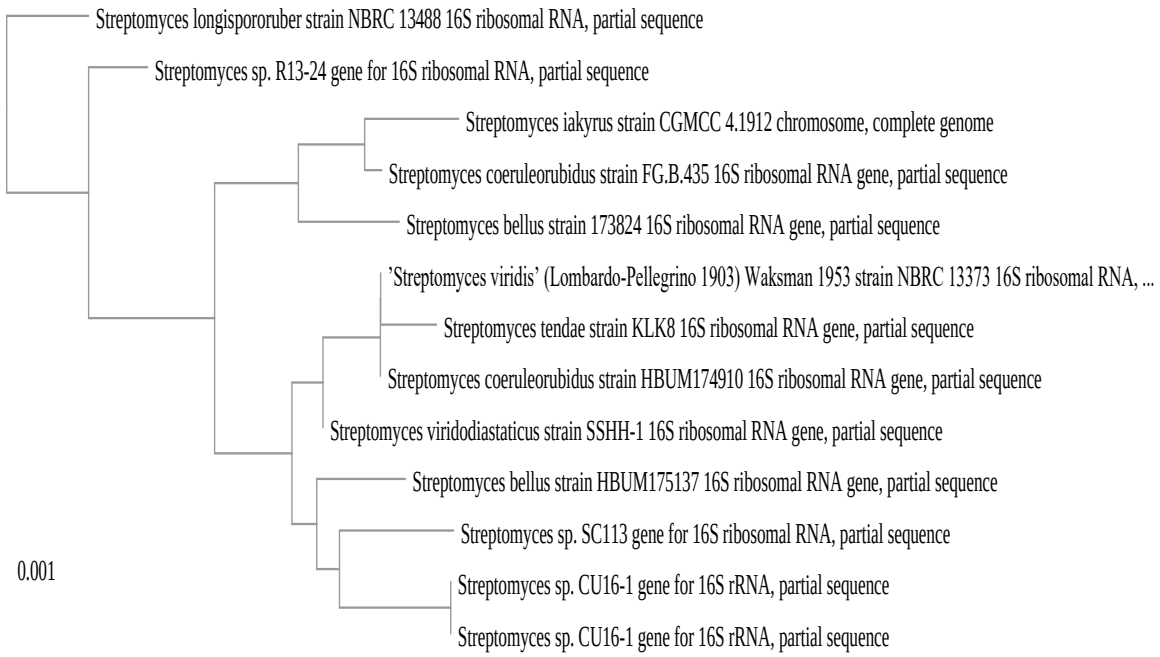

Figure (3): Phylogram of *Streptomyces* sp. CU16-1 gene for 16SrRNA sequences on NCBI.

3-4- Direct DNA Isolation from the Soil

From the soil, 8 samples were taken, and after conducting direct extraction of DNA and examining DNA purity examination with the nanodrop, most of the samples showed purity and good concentrations of DNA, ranging in purity between 1.6-2, meaning that the DNA was purified without contamination with humic substances in different soil samples, although there is no agreement on an effective purification method [30]. As pure soil samples give the best results in molecular techniques [9]. Organic matter is the main source of inhibitors for

directly extracted microbial DNA; in particular, humic acids are a major problem and will interfere with enzymatic manipulation of DNA [31,32]. So, the use of glass beads within the extraction protocol was important in the destruction of bacterial cells and DNA extraction with a degradation efficiency of more than 90% [33]. As traditional methods of growing and purifying isolates in special media may be expensive and stressful when treating a large number of isolates [34,35].

3-5-Polymerase Chain Reaction (PCR)

A chain amplification reaction was performed using the same primers used in the traditional method on eight soil samples, as one sample showed a positive result, but the other seven samples showed a negative result, and this may be due to the fact that the weight taken from the soil sample for direct extraction of DNA does not contain *Streptomyces*, or there may be technical reasons related to the work of PCR. The emergence of a positive result for one of the samples is an important step in the field of molecular technology for bacterial species in general and *Streptomyces* in particular by directing research and focusing on extraction methods. DNA to rapid mechanisms and avoid intensive purification steps [36].

Using bead grinding mechanisms, it is possible to extract a variety of DNA from different microbes, and the mechanism used in this research showed a positive result, although it was one sample out of eight samples, but it proved the success of extraction methods, and the method can be adopted in the future, and molecular microbial techniques can be applied more easily.

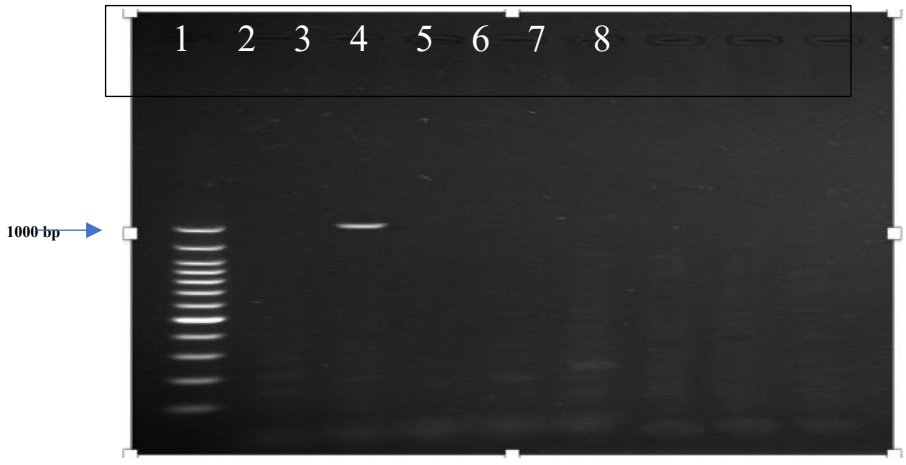

Figure (4): Agarose gel electrophoresis of PCR amplification products of genomic DNA isolated directly from soil: lane L, DNA ladder1000bp; lane 2 showed *Streptomyces* isolate, while lanes 1, 3, 4, 5, 6, 7, 8 did not show a *Streptomyces* isolate

3-6- Real-Time Polymerase Chain Reaction (qPCR)

Real-time PCR was performed on the eight samples by used Primers F-(FWD)5'-TCAAAAAGCCGGTCTCAGTTC-3'and R-(REV) 5'-GGTGTTACCGACTTTCGTGAC- 3' newly designed based on accession no. NR_116013 by 1450bp size with a size of 1450 bp targeting 16SrRNA that were their DNA directly extracted from soil and showed a positive result for six out of eight samples, which proved that *Streptomyces* are present in all types of soil and belong to the Actinobacteria phylum which is considered as the second largest bacterial phylum in the soil [37]. While the result showed negative for two samples, which the reason may be that the increased DNA concentration in the sample did not allow the primers to bind to the DNA pieces, which caused confusion for the qPCR readings. The appearance of the positive result for most direct extraction isolates showed the success of the direct extraction process, which is a quick important method, as accurate quantification cannot be reliable using standard PCR and the technique has been improved by qPCR, which gives the powerful analytical tool and results at the time of the reaction itself, so it is called qPCR [38]. Apart from providing great accuracy and specificity, qPCR relies on qualitative and quantitative analysis as it has become the preferred technology for rapid and accurate detection and measurement of DNA in many biological samples for many diverse applications such as gene expression analysis, identification and quantification of bacterial species, microRNA analysis, detection of genetically-modified organisms, and many other important applications due to for its diversity and usefulness [39.40].

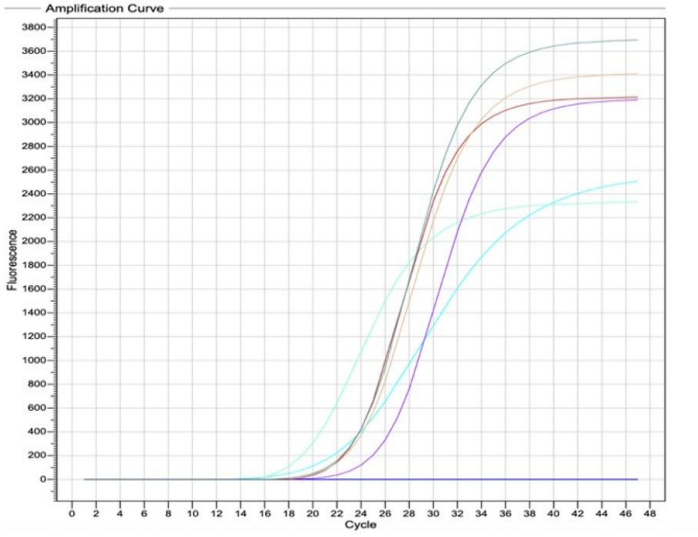

Figure (5): Shows the amplification curve of the eight isolates during qPCR analysis and shows the cycle number in which the fluorescence was performed for each isolate.

Table (1): Shows the results of the quantitative analysis of the multiplication process and the cycle threshold values Ct at which a detectable signal is reached. A lower Ct indicates a higher abundance in the sample, while a higher Ct indicates a lower number of spores in the sample

#	Well	Assay Item	Property	Dye	Ct	Ct Aver.	Ct SD	Cal. Con.	Con. Aver.	Con. SD	Sample ID
1	A01	16S rRNA	Unknown	SYBR	24.11	24.11	0				16S rRNA-1
2	A02	16S rRNA	Unknown	SYBR	20.75	20.75	0				16S rRNA5
3	A03	16S rRNA	Unknown	SYBR		0	0				16S rRNA12
4	A04	16S rRNA	Unknown	SYBR		0	0				16S rRNA18
5	A05	16S rRNA	Unknown	SYBR	26.51	26.51	0				16S rRNA6
6	A06	16S rRNA	Unknown	SYBR	23.96	23.96	0				16S rRNA17
7	A07	16S rRNA	Unknown	SYBR	24.27	24.27	0				16S rRNA19
8	A08	16S rRNA	Unknown	SYBR	23.96	23.96	0				16S rRNA34

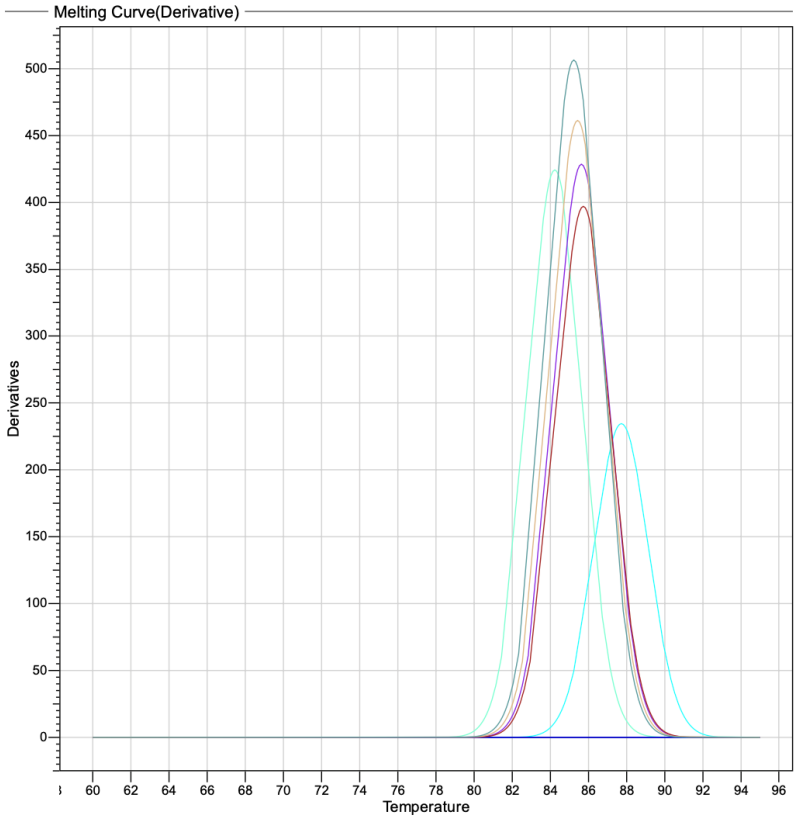

Figure (6): Shows the melting curves of the isolates in the derivative phase, which shows the temperatures and fluorescence for each isolate.

Table (2): Showing the melting results of the eight isolates which had DNA extracted directly in the qPCR, which shows a negative result for two of samples 3 and 8, while showing a positive result for six isolates. No. 10 and No. 12 are positive control and negative control respectively, and No. 11 is 16SrRNA blank.

#	Well	Assay Item	Property	Dye	Ct	Ct Aver.	Ct SD	Cal. Con.	Con. Aver.	Con. SD	Sample ID
1	A01	16S rRNA	Unknown	SYBR		0	0				16S rRNA-1
2	A02	16S rRNA	Unknown	SYBR	13.32	13.32	0				16S rRNA-2
3	A03	16S rRNA	Unknown	SYBR		0	0				16S rRNA-3
4	A04	16S rRNA	Unknown	SYBR	19.49	19.49	0				16S rRNA-4
5	A05	16S rRNA	Unknown	SYBR	26.74	26.74	0				16S rRNA-5
6	A06	16S rRNA	Unknown	SYBR	17.23	17.23	0				16S rRNA-6
7	A07	16S rRNA	Unknown	SYBR	16.52	16.52	0				16S rRNA-7
8	A08	16S rRNA	Unknown	SYBR		0	0				16S rRNA-8
9	A09	16S rRNA	Unknown	SYBR	16.94	16.94	0				16S rRNA-9
10	A10	16S rRNA	Unknown	SYBR	20	20	0				16S rRNA-PC
11	A11	16S rRNA	Unknown	SYBR		0	0				16S rRNA-BLANK
12	A12	16S rRNA	Unknown	SYBR		0	0				16S rRNA-NC

3-7-Statistical analysis of PCR and qPCR results

Statistical analysis of PCR and qPCR reading data was performed to verify whether the direct method of DNA isolation using qPCR amplification chain reaction is statistically valuable, and through (table 3), it showed a p-value of less than 0.05, which indicates that the differences between the arithmetic averages are statistically significant in favor of qPCR and according to the average values for each type and thus prove the success of using PCR-RT samples for direct isolation of DNA and shortening time and effort with accurate results. Fig. 7 also shows the values of the qPCR average, which is higher than the PCR average.

Table(3): Statistical analysis of PCR and qPCR results showing a higher qPCR average than the PCR average for positive values in both cases, which shows a value of df degrees of freedom and a value of sig(2-tailed) less than 0.05

Type of PCR	N	Mean	t	df	Sig. (2-tailed)	Mean difference	95% confidence interval of the difference	
qPCR	8	7500	3.035	14	0.009	0.62500	lower	Upper
PCR	8	50					0.18331	1.06669

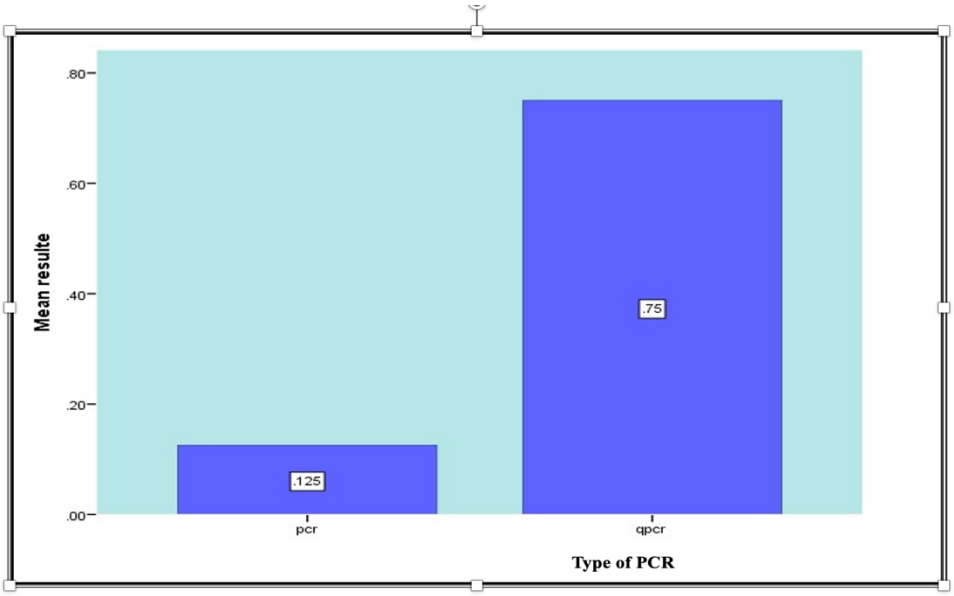

Figure (7): Statistical analysis of PCR and qPCR results

4− CONCLUSIONS

Our study highlighted the possibility of isolating *Streptomyces* bacteria from different soil samples and the possibility of isolating DNA directly from the soil, which is an important step in shortening the effort and time to isolate new strains of these bacteria, benefits in the medical field and the agricultural field for their production of many biologically active substances, so conducting more studies on direct isolation could expand the discovery of new species and strains that have the ability to produce new bioactive substances that may have a significant impact on the development of many medical and agricultural fields.

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