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Cultural, Microscopic, and Genetic Identification of a Natamycin-Producing Streptomyces Isolate

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ABSTRACT: Soil samples were collected from six different fields, including those at the University of Baghdad/Al-Jadriya, Abu Ghraib, Zakho, Sulaimaniyah, Kirkuk, and Duhok, and were assigned the following codes: G, B, Z, S, K, and D, respectively. The samples were divided into two groups. One group was treated with calcium bicarbonate (10 g soil / 1 g calcium carbonate), while the other group was heated (56°C / 30 minutes). After performing decimal dilutions, the samples were cultured on three selective solid culture media for Streptomyces bacteria: Starch casein agar (SCA), glycerol asparagine agar (GAA), and tyrosine agar (TA), in addition to the general culture medium, Nutrient agar. The soil samples collected from the Abu Ghraib field performed better than the other samples tested from the five fields. The second treatment method for the soil samples was superior to the first, and the selective culture medium, SCA, outperformed the other three in obtaining a higher number of colonies. Nineteen colonies were collected with culture characteristics similar to those of Streptomyces: seven colonies designated B, four G, three D, two K, two S, and one Z. Based on culture characteristics such as the slightly grayish-white color, height, and characteristic earthy odor (gusmine) of Streptomyces, initial screening was completed by culturing the collected colonies on glass slides using slide culture. Six isolates were selected from among the nineteen that exhibited clear branching filaments under the microscope and a positive Gram stain (violet). These six were then selected for the second screening stage, which involved testing the isolates' ability to produce natamycin in Three liquid culture media, SCB, GAB, and NB, were tested. Isolate B4 exhibited the highest natamycin yield when cultured on SCB liquid medium, surpassing both tested media and the other tested isolates, with a yield of 557.50 mg/ml. isolate B4 was selected and subjected to genetic diagnosis based on 16S rRNA , It was found to belong to Streptomyces grisues with a percentage match.

INTRODUCTION

This genus is the largest taxonomic group within the bacterial domain. It is a member of Actinobacteria, which comprises the largest number of species in the order Actinomycetales. More than 650 species are found in the German Collection of Microorganisms and Cell Cultures (DSMZ) (**Stackebrand, Rainey, 1997**). Based on these classifications, the genus Streptomyces is divided into 20 major groups, 41 subgroups, and 22 monogroups. Major groups are defined as groups of species consisting of six or more strains, while minor groups are defined as single species consisting of two to five strains (**William *et al.*, 1983; Stackebrand, Rainey, 1997).** This group is characterized by a strong propensity to produce antibiotics with diverse chemical structures and biological activities (**De Lima *et al.*, 2012).** They represent valuable resources for discovering active metabolites with diverse applications. Most antibiotics originated from various species of the genus Streptomyces **(Clardy *et al.*, 2006).** The genus Streptomyces is the most abundant in soil among the actinomycetes, are most

abundant in acidic soils, particularly acid-resistant groups, while they are less abundant in alkaline soils (**Poomthongdee *et al.*, 2015**). Their availability is particularly high in soil, where they constitute between 1% and 20% of the total number of living bacteria (**Kämpfer, 2012; Kumar and Jadeia,** 2016), although they are widespread in aquatic and terrestrial environments (Kämpfer, 2012; Barka, 2016). Streptomycete availability, like that of microbial communities, is influenced by biotic and abiotic factors, especially soil type, vegetation cover, and climate (**Singh and Raim, 2004).** The omponents of the medium influence the isolation of Streptomyces (**De Simeis and Serra 2021**). It has been observed that the best isolation occurs in media containing starch or glycerol as the carbon source, and casein, arginine, or nitrate as the nitrogen source. Various antifungal agents, such as nystatin, cycloheximide, or albemaric acid, are commonly used during the isolation process to obtain pure bacterial isolates. Streptomyces bacteria are identified by the size, shape, chains, pigmentation, physiological and biochemical characteristics, and antibiotic resistance of their spores (Rashed *et al.*, 2015). Kumar *et al.*, (2010) also obtained a pure colony in the isolation of Streptomyces sp. using starch-casein agar (SCAM). Yeast extract-malt (YMG) medium is also useful for Streptomyces isolation, both for primary culture and for bacterial growth. **The** traditional classification of the Streptomyces genus is based on phenotypic characteristics, pigment production, and some physiological characteristics. Traditional identification of bacteria based on phenotypic characteristics is not as accurate as identification based on genetic methods (**Clarridge, 2004**). A new criterion for bacterial identification, which differs from the classical approach, began to be developed in the 1980s. It is based on comparing the sequence of bacterial 16S rRNA genes. 16S rRNA sequences have provided actinomycetes with a phylogenetic tree that provides the basis for their identification and enables the study of actinomycetes' evolution (**Sivakumar *et al.*, 2004).** They are living organisms that reproduce by means of spores residing at the ends of aerial fungal hyphae (Sharma, 2014). Colonies are initially soft and tender, then become hard and cohesive, granular, powdery, cottony, or velvety. Most strains can produce species-specific antibiotics (**De Lima *et al.*, 2012**). They are aerobic, Gram-positive, and non-motile (**Tanaka and Omura 1990).** Streptomycetes are known for their characteristic earthy odor, which results from the production of a volatile metabolite called geosmin **(Justine,2021).**

MATERIALS AND METHODS

Soil sample collection: Six soil samples were collected from different locations, including fields in Baghdad (Al-Jadriya and Abu Ghraib areas), as well as fields in Zakho, Dohuk, Kirkuk, and Sulaymaniyah. A 3 cm layer of soil was scraped, and six samples were taken at a depth of 10 cm and another six at a depth of 20 cm, depending on subsequent soil treatment. The samples were then placed in polyethylene bags, sealed tightly, and labeled with the sample number and code G, B, Z, D, K, and S, respectively, along with the location from which they were collected. The samples were then transported to the laboratory and refrigerated until testing and sample preparation. The pH of the soil samples was determined using the 1:5 soil-water method, as described by the **FAO (2021).**

preparation of samples: Soil samples were treated to prepare them for isolation and sieving. The samples were treated in two ways, as the soil samples were divided into two groups, each group containing six samples (collected from the same site but using a different collection method): **A-The first treatment:** This included a group of six soil samples taken at a depth of 20 cm, which were treated with calcium carbonate CaCO3 (10 g soil: 1 calcium carbonate) and placed in an incubator at 37°C for 4 days. After that, a series of dilutions were carried out for each sample, starting from 10^{-1} to 10^{-5}, by suspending the soil in peptone water. 0.1 ml of each of the last three dilutions was transferred to dishes containing the following culture media:

1- Nutrient Agar (NA)
2- Starch Casein Agar (SCA)
3- Glycerol Asparagine Agar (GAA)
4- Tyrosine Agar (TA)

All the plates were placed in the incubator at 28°C for seven days (**Balagurunathan and Subramanian,2001).**

B- The second treatment: For the other six soil samples taken at a depth of 10 cm, as mentioned by **Ban *et al.*, (2023),** the soil samples are dried overnight in a chemical hood and then heated in a water bath for 30 minutes at 56°C. After that, the decimal dilutions are carried out with sterile distilled water (10^{-1} to 10^{-5}). 0.1 ml of each of the last three dilutions is transferred to plates containing the four culture media mentioned above, as in the first treatment.

Isolation and screening of Streptomyces colonies

1- Initial screening: It was based on the culture characteristics of the isolates on four different culture media, in addition to the microscopic morphological characteristics. The culture was performed using the pour-plate method with four solid culture media to increase the selectivity for obtaining Streptomyces colonies. The plates were then incubated at 28°C for 7 days. The isolation and sifting process continued. **1- By selecting typical colonies** that possess the characteristic agricultural properties in terms of shape, color that changes according to the culture medium, their distinctive earthy smell, and their chalky appearance. Then the culture process was repeated individually for each colony selected using the Loop microbial transfer tool on the best solid culture medium, which is determined by two replicates for each colony, with the aim of obtaining pure colonies (**Zeynep *et al.*,2022; Jhon and Fresthel,2022 ; Zahraa,2013**). **2- Slide Culture** to determine the complete morphology of Streptomyces (its bacilli with hyphae) under a light microscope, a glass slide is used, placed on a glass rod in a V-shape inside a glass dish that is well covered with aluminum foil and sterilized in an autoclave (121°C, 20 minutes), pour 2 ml of Starch Casein agar and Glycerol Asparagine Agar medium onto the slide while it is inside the dish. Re-cover the dish and place it at a slight angle. Then leave it until the medium solidifies. After that, culture is done on the glass slide by transferring a part of the colony selected according to the agricultural specifications from the previous step using a needle onto the culture medium and cover it with a glass slide cover slip. Place the plate in the incubator (28°C, 7 days). Remove the slide and examine it under a light microscope at 10X magnification and then 40X (**Nidhal and Asmaa, 2025**).

Microscopic characteristics: The selected colonies were examined microscopically by Gram staining to determine their response to G+ or G- staining, and the shape and arrangement of their cells, hyphae, and cell structure, using a compound light microscope under a 10X microlens and then a 100X oil lens (**Harrigan and MacCance, 1976**).

Second screening of isolates by testing their productivity for natamycin

A second screening was performed on the isolates selected in the initial screening stage. 300 ml flasks containing 100 ml of liquid production medium (starch casein broth) were inoculated with 1 ml of Streptomyces suspension from each isolate individually. The flasks were then placed in a shaker incubator at 180 rpm for 7 days at 28°C (**Pulak *et al.*, 2020**). Following this, centrifugation was performed for 20 minutes at 5000 rpm. The supernatant (bacterial filtrate) was used for subsequent tests to determine the amount of natamycin and identify the most efficient isolate in terms of natamycin production.

Genetic identification of the most efficient isolate

Genetic identification was performed at the Scientific Progress Office – Baghdad – Al-Harithiya. (the Presto Mini Gdna Bacteria) Kit, supplied by Geneaid Company – Taiwan, was used to extract DNA. Identifying and analyzing nitrogenous base sequences: The amplification products were sent to the Korean company Macrogen to determine the nitrogenous base sequences, along with the available information about the 16Sr RNA gene in the National Center for Biotechnology Information (NCBI) gene bank, and according to the BLAST Nucleotide program to identify the selected isolate species.

RESULTS AND DISCUSSION

The isolation environment is the cornerstone of the isolation process and the selection of the required isolate in any type of microbial bioproduction. Soil is the primary reservoir for isolating microorganisms, but the type of soil varies according to the location and prevailing environmental conditions, and this results in a different type of microbial load for the soil. This is what we found in our experiment to isolate Streptomyces bacteria from soils of six fields in different locations (Table 1). Nineteen colonies were collected from the six sources, and among them, the soil sample from a field in Abu Ghraib was distinguished by having the highest number of colonies, reaching 7, according to the culture specifications for Streptomyces. This was followed by the soil from the Al-Jadriya Gardens field at the University of Baghdad with 4 colonies, then the Dohuk field with 3 colonies, followed by the Kirkuk field with 2 colonies, 2 colonies from a field in Sulaymaniyah, and 1 colony from a field in Zakho. The following symbols were assigned to these soils: B, G, D, K, S, and Z, respectively.

Table (1) Location and environmental characteristics of Streptomyces isolation sources

	Collection site / symbol	pH	Soil color	Soil nature
1	University of Baghdad / Al-Jadriya **G**	alkaline	brown	clay
2	Abu Ghraib / Baghdad **B**	mild alkaline	brownish-gray	heavy clay

3	Zakho / Northern Iraq **Z**	alkaline	dark to medium brown	Mixed
4	Kirkuk / Northern Iraq **K**	alkaline	Yellowish-brown	clayey to oily
5	Sulaymaniyah / Northern Iraq **S**	alkaline	dark brown to dark brown	Mixed
6	Duhok / Northern Iraq **D**	alkaline	Light brown	Sandy (dry)

The number of Streptomyces isolates varies depending on the soil type. **Mahima *et al.*, (2021)** collected the largest number of Streptomyces isolates from a landfill compared to those collected from forest soil, and more isolates from slightly acidic to alkaline soils at temperatures between 29°C and 33°C. Three selective culture media for Streptomyces—SCA, GAA, and TA—were used, in addition to the general medium NA, to increase the chances of isolating and obtaining them from the microbial community present in their environment. SCA medium proved to be the most selective for Streptomyces. While the second treatment of the soil samples was more suitable for isolating the bacteria in the current study than the first method, this may be because the first method treated the soil with calcium carbonate, which alters the soil's acidity, and Streptomyces tends to grow in a slightly alkaline medium. In the second method, the soil samples were heated to 56°C for half an hour.

This affects the vegetative cells of microscopic species and increases the production of streptomycetes. Soil type is an influential factor in the nature of the species found in it; our results are consistent with those indicated by **Poomthongdee *et al.*, (2015)** Streptomycetes, which belong to the Actinomycetes group, are more abundant in the soil and are greatly affected by physical and chemical conditions such as the types of organic matter, temperature, soil moisture content and pH. The acid-resistant members of the group are associated with acidic soils, but less so with alkaline soils.Fiigure (1) illustrates that the best soil source and the best selective medium for Streptomyces in our experiment was casein agar culture medium. We note that storing the soil for a few days in a laboratory atmosphere and then drying it resulted in treatment one being better than treatment two. This may be due to the reduction of soil moisture first, providing a greater opportunity for selection of Streptomyces bacteria from the bacterial population. This was also found by **Jhon and Fresthel (2022)** in their experiment isolating Streptomyces from different soils in Calaba, Philippines.

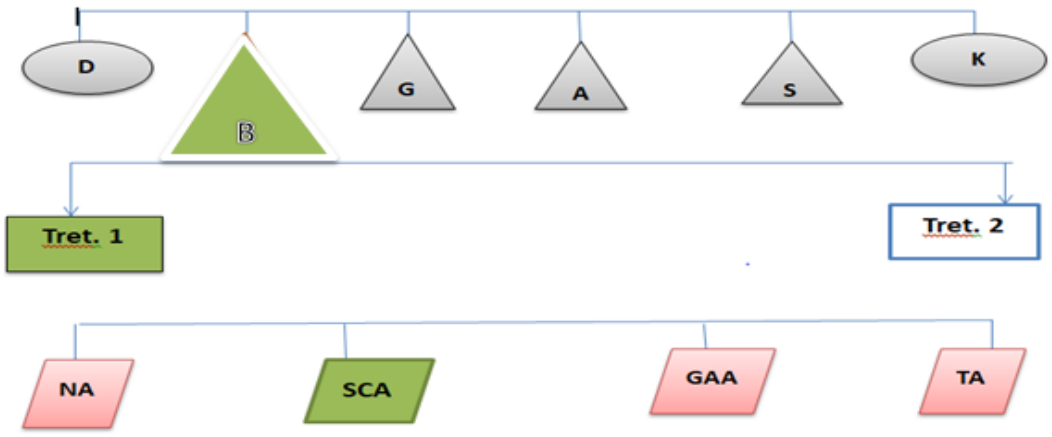

Fig (1) The source of the soil, the treatment, and the solid culture medium that was chosen are shown.

Agricultural characteristics

The culture characteristics of nineteen selected colonies from the chosen culture media were studied, and six colonies were selected based on these characteristics. One colony appeared as a small, circular, grayish-white colony with a slightly wavy, circular edge and a distinct earthy odor, distinguishing it from the SCA culture medium. It was given the symbol (AH). Colony (B3) also appeared as a circular, red colony.

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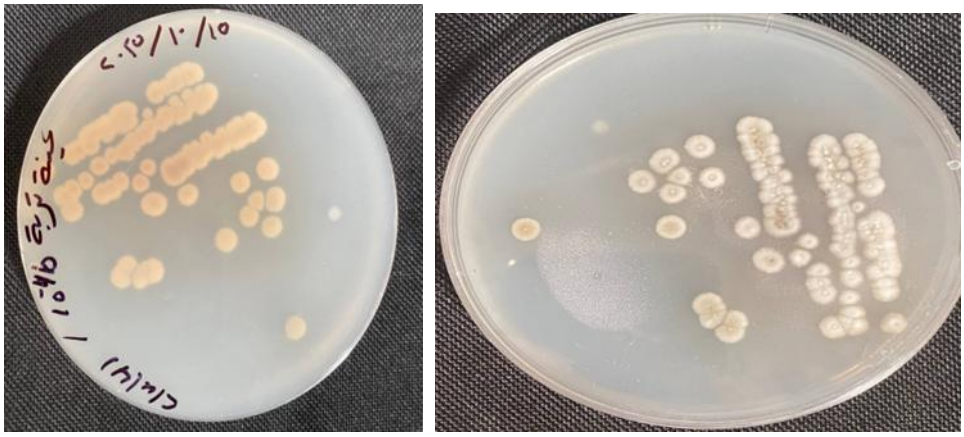
When colony (B2) was on the same medium, it was slightly raised above the culture medium, round, punctate, swollen, and black in color. Colony (B1) was also raised above the medium, white to gray in color, with an earthy smell, and its edge was slightly wavy. Colony (B4) was also chosen; it was not raised above the medium but was flat, light gray in color, with a slightly wavy edge and a chalky appearance. Colony (G) appeared round, white in color, small in size, and not raised above the medium. These results are consistent with what **Zahraa (2013**) observed in her characterization of Streptomyces colonies isolated from the soil. She obtained good growth on GAA culture medium with a gray and white color and white mycelium without pigments, and also good growth on starch mineral salt agar medium with a gray, white, green and red color, without mycelium and pigments. The growth was moderate with a red color and intermediate pigments with no mycelium on peptone glycerol yeast extract agar medium, and the same for tyrosine agar medium in terms of the nature of growth and pigments, but the colonies were white and gray with brown and white mycelium.



B **A**

Fig (2) Cultural characteristics of selected isolate colonies grown on SCA culture medium (A : Isolate B4, B : Isolate B3)

The culture characteristics, in terms of chalky appearance and earthy odor, of the colonies we collected from the selective medium SCA are consistent with **Zahraa (2013**) study of the culture morphological characteristics of Streptomyces isolates. Other colors besides white with a grayish tinge were observed in Streptomyces colonies. Our results are consistent with those obtained by **John and Fresthel (2022)** when isolating Streptomyces from Calamba City in the Philippines, where more than half of the colonies were grayish-white. The type of culture medium is an influential factor; the colors of the fungal hyphae differ on their anterior and posterior sides, as in the case of *S.peucetius* , this species differs from other species in the color of the mycelium on the aerial side and the posterior side when grown on Glycerol arginine medium (GAM) (**Zeynep *et al.*, 2022**). SCA culture medium is a preferred medium for Streptomyces growth and was our preferred choice in our study. It contains starch and casein, which represent the carbon source and energy sources, and are complex multi-components that not every microorganism is able to use as carbon sources. Most molds and Streptomyces are able to grow in this medium (**Ajijua, 2008).** The results of the initial screening experiment show that the solid medium SCB is superior to the two solid culture media used in our experiment for obtaining pure and single colonies (fig.2). This is consistent with what **Kumar *et al.*, (2010)** found when they isolated Streptomyces that the best medium was SCA and Malt extract glucose medium (YMG) for culture growth and pre-culture. The best medium for isolating Streptomyces bacteria is when starch or glycerol is the carbon source, and casein or arginine is the nitrogen source (**Rashad *et al.*, 2015**). To facilitate the discovery of microorganisms that produce beneficial natural metabolites, the role of the culture medium in this process is highlighted.

Microscopic characteristic

A) Selected isolates were stained with Gram stain to identify their cell shape and arrangement, as well as their reaction to Gram stain. Under a light microscope, all isolates subjected to this staining method showed a positive reaction to the stain and a rod-shaped morphology, some appearing as short rods interspersed with thin filaments resembling fungal filaments but smaller in diameter, such as isolates B3, B4, B1, and G2. Isolates that did not exhibit hyphae under the microscope were excluded to shorten the steps in the screening and isolation process to reach the desired type. Figure (3) shows the cells of isolate (B4) after staining with Gram stain.

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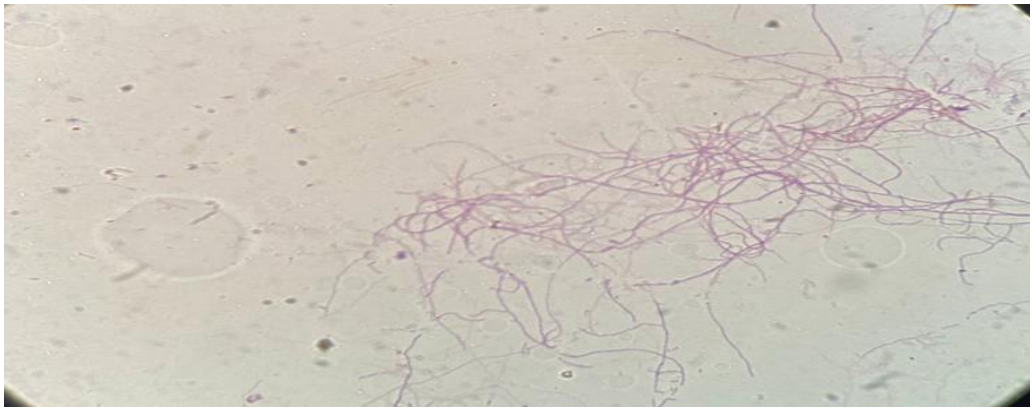

Fig (3) B4 isolate cells under a light microscope (100X) stained with Gram stain

B) Cultivation on a glass slide: Culture of fungi on a glass slide is used to see the entire mold body (mycelium) and to accurately identify hyphae, since Streptomyces bacteria form hyphae, a characteristic common to fungi and a distinguishing feature, as shown in Figure (4) hyphae on the culture medium poured onto the slide under a light microscope. Streptomyces bacteria are identified by the size, shape, and arrangement of their spore chains, in addition to the pigments they form and their biochemical properties **(Rashad *et al.*, 2015).**

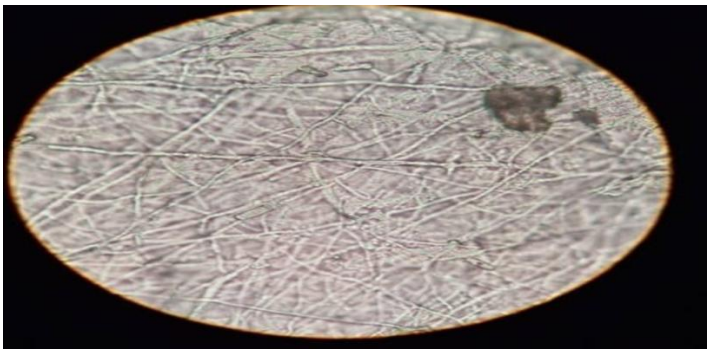

Fig (4) Isolation B4, light microscopy (40X), cultured on SCA medium using the Slid culture method

Second Screening : Isolate B4 showed the highest natamycin production compared to the other tested isolates, with its highest productivity observed on SCB medium. with a yield of 557.50 mg/ml.

Genetic identification of the selected isolate

Isolate B4 was chosen because it yielded the highest amount of natamycin among the five isolates tested with it, and subsequent experiments in the current study were conducted with it. The diagnosis relies on the 16S rRNA gene. The purity of the extracted genetic material was confirmed by measuring the absorbance at 260 and 280 nm. Electrophoresis of the amplified gene showed a single band (figure 5) for the selected bacterial isolate, reflecting successful binding of the target gene (16S rRNA) to the primers without affecting the other parts of the DNA extract from the selected isolate. The molecular size of the amplified product was 570 base pairs. The 16S rRNA gene provides conclusive results in identifying bacterial species and is successful in differentiating between them (Lakshmi *et al.*, 2014).

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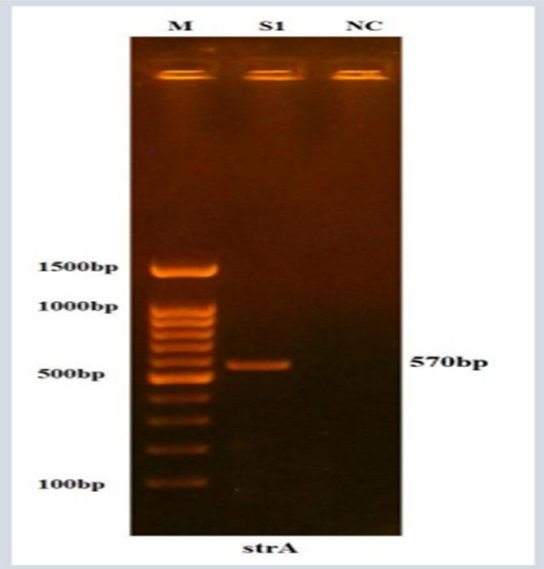

Fig (5) Electrophoresis of selected isolation B4 on agarose gel with a volumetric index of 500-1500 base pairs

Analysis of the amplification product sequences

The 16S rRNA gene sequences of the selected isolate were studied. After performing analysis using Blast software to find genetic similarity with the NCBI GenBank, the results showed a 100% match in the 16S rRNA gene sequences of isolate B4 with the nucleotide sequences of the *Streptomyces griseus* CB147 strain registered with the NCBI, as shown in Table (2). Thus, the selected bacterial isolate was considered to be Streptomyces griseus. The process of genetic manipulation of Streptomyces species began with the isolation of genomic DNA, which is used for sequencing, gene amplification, building genomic libraries, and a variety of other protocols. Information obtained from genomic DNA forms the basis for more advanced gene manipulation experiments, such as gene cluster isolation and metabolic pathway engineering.

Table (2) Nitrogenous base sequences of the 16S rRNA gene for local isolate B4

Score	Expect	Identities	Gaps	Strand
819 bits(443)	0.0	487/509(96%)	0/509(0%)	Plus/Plus

```
Query  1    TCGACGGAGCCCGCACCCTGGCGTCGATCGATGATGACGACGTGGCGATGGGCATCCTCG  60
Sbjct  26   ............................C............................  85

Query  61   CCGGACTGCACGCGCGGCTGGTCGCGGTCCCCGCGCCGCAGGGGCTGCGCGGCCTCGGTG  120
Sbjct  86   ....C.......................................................  145

Query  121  AGGTCGCCGCCGCGATGCTGGAGCAGGTGCCGCAGGTGGTCACTCATCTGACGGACCCGG  180
Sbjct  146  ............................C.......C......C..C.............  205

Query  181  CCGACCGGGATCTGCTGCGGGGCTGGGCATCGGCCGTCGCCGAACTGGCCGACGAGCCCG  240
Sbjct  206  ..........C.................................................  265

Query  241  GAGACCGGTTGCTGCACTGGGATCTCCACTACGACAACGTCCTTGCCGCGCAGCGTGAGC  300
Sbjct  266  ..................................................CG.........  325

Query  301  CGTGGATCGCCATCGACCCCGAGCCGCTCGCCGGTGATCCGGGGTTCGACCTGTGGCCCG  360
Sbjct  326  ......................A..........................................  385

Query  361  CCCTGGACAGCCGGTGGGACGACGTCGTTGCGAAGGGCGACCCGCTGCGTGTGGTGCGCC  420
Sbjct  386  ............................C..............A.......G....G...  445

Query  421  GCCGGTTCGACCTGCTGACGGACACCCTCGGTCTGGACCGCGCCCGGGCTGCCGGCTGGA  480
Sbjct  446  ................C..C...G.T.....C...........G.....C...........  505

Query  481  CGCTGGGGCGGCTGCTGCAGAACGCCCTG  509
Sbjct  506  ..............T..............  534
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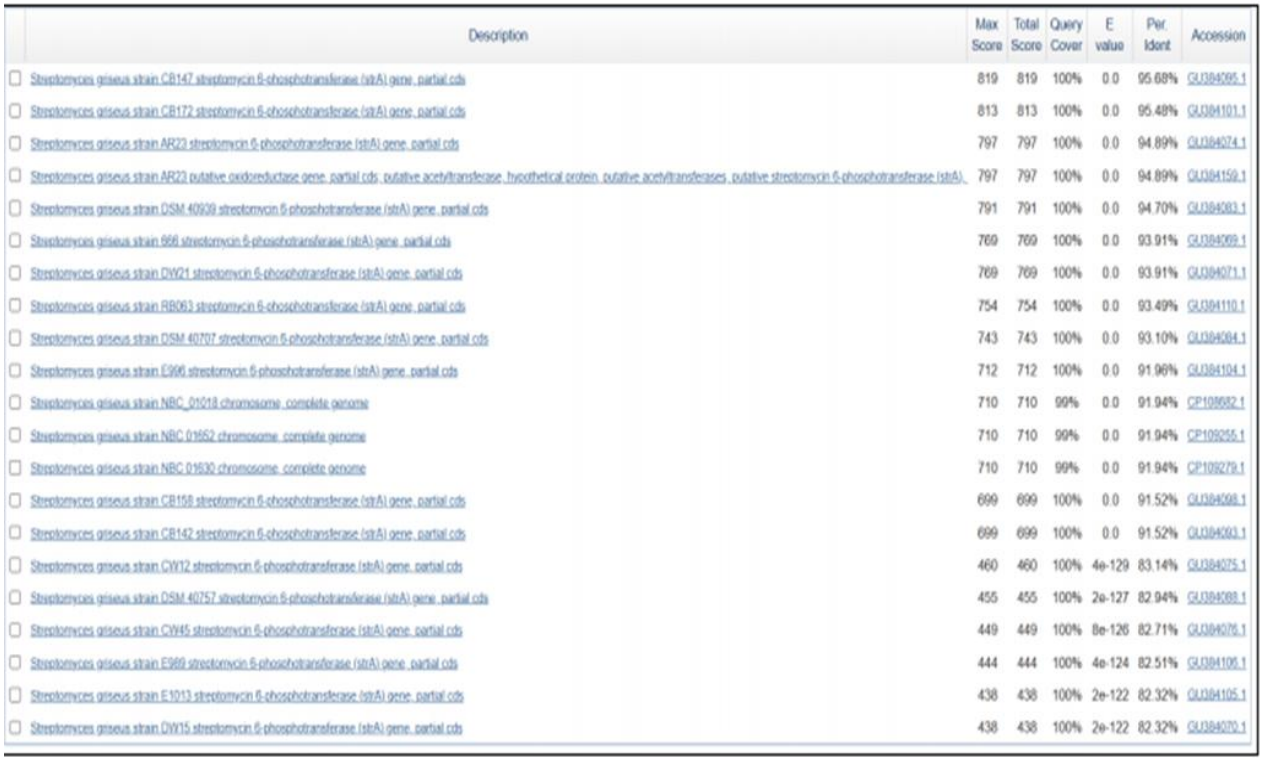
Omar *et al.*, (2015) described the Neighbor-Joining method for inferring the evolutionary history of Streptomyces strains. To amplify the 16S rRNA gene, the primers 785F-5'GGATTAGATACCCTG GTA3' and 907R-5'CCCGTCAATTCMTTTRAGTTT 3' were used, and the PCR amplification products were sequenced. The phylogenetic tree was constructed using MEGA X software **(Kumar *et al.*, 2018**). Some statistical values from the Blast program, such as Score and Expect values, are generally considered indicators of genetic similarity based on nitrogenous bases. The phylogenetic tree between the isolates was constructed using MEGA6 software based on the 16S rRNA gene sequences. The program employs the most probable ratio.

To find the genetic relationship tree to test the most accurate probability of the degree of evolutionary relatedness between the isolates **(Tamura *et al.*, 2013**). As **Figure (6)** shows the Score and Expects values that indicate some statistical values specific to the BLAST program, note that the score value is 819 and the Expect value is (zero). The higher the Expect value from zero, the more it gives indications of an increased degree of congruence between the bacterial isolate (selected) under study and the sequences of nitrogenous bases in the gene bank of bacteria related to it.



Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Streptomyces griseus strain CB147 streptomycin 6-phosphotransferase (strA) gene, partial cds	819	819	100%	0.0	95.68%	GU384095.1
Streptomyces griseus strain CB172 streptomycin 6-phosphotransferase (strA) gene, partial cds	813	813	100%	0.0	95.48%	GU384101.1
Streptomyces griseus strain AR23 streptomycin 6-phosphotransferase (strA) gene, partial cds	797	797	100%	0.0	94.89%	GU384074.1
Streptomyces griseus strain AR23 putative oxidoreductase gene, partial cds; putative acetyltransferase, hypothetical protein, putative acetyltransferases, putative streptomycin 6-phosphotransferase (strA),	797	797	100%	0.0	94.89%	GU384159.1
Streptomyces griseus strain DSM 40939 streptomycin 6-phosphotransferase (strA) gene, partial cds	791	791	100%	0.0	94.70%	GU384083.1
Streptomyces griseus strain 966 streptomycin 6-phosphotransferase (strA) gene, partial cds	769	769	100%	0.0	93.91%	GU384069.1
Streptomyces griseus strain DW21 streptomycin 6-phosphotransferase (strA) gene, partial cds	769	769	100%	0.0	93.91%	GU384071.1
Streptomyces griseus strain RB063 streptomycin 6-phosphotransferase (strA) gene, partial cds	754	754	100%	0.0	93.49%	GU384110.1
Streptomyces griseus strain DSM 40707 streptomycin 6-phosphotransferase (strA) gene, partial cds	743	743	100%	0.0	93.10%	GU384084.1
Streptomyces griseus strain E996 streptomycin 6-phosphotransferase (strA) gene, partial cds	712	712	100%	0.0	91.96%	GU384104.1
Streptomyces griseus strain NBC_01018 chromosome, complete genome	710	710	99%	0.0	91.94%	CP109582.1
Streptomyces griseus strain NBC 01652 chromosome, complete genome	710	710	99%	0.0	91.94%	CP109255.1
Streptomyces griseus strain NBC 01630 chromosome, complete genome	710	710	99%	0.0	91.94%	CP109279.1
Streptomyces griseus strain CB158 streptomycin 6-phosphotransferase (strA) gene, partial cds	699	699	100%	0.0	91.52%	GU384098.1
Streptomyces griseus strain CB142 streptomycin 6-phosphotransferase (strA) gene, partial cds	699	699	100%	0.0	91.52%	GU384093.1
Streptomyces griseus strain CW12 streptomycin 6-phosphotransferase (strA) gene, partial cds	460	460	100%	4e-129	83.14%	GU384075.1
Streptomyces griseus strain DSM 40757 streptomycin 6-phosphotransferase (strA) gene, partial cds	455	455	100%	2e-127	82.94%	GU384088.1
Streptomyces griseus strain CW45 streptomycin 6-phosphotransferase (strA) gene, partial cds	449	449	100%	8e-126	82.71%	GU384076.1
Streptomyces griseus strain E989 streptomycin 6-phosphotransferase (strA) gene, partial cds	444	444	100%	4e-124	82.51%	GU384106.1
Streptomyces griseus strain E1013 streptomycin 6-phosphotransferase (strA) gene, partial cds	438	438	100%	2e-122	82.32%	GU384105.1
Streptomyces griseus strain DW15 streptomycin 6-phosphotransferase (strA) gene, partial cds	438	438	100%	2e-122	82.32%	GU384070.1

Fig (6) Score and Expect values for the Streptomyces isolate under study and the bacteria registered in the gene bank

The *Streptomyces griseus* genome consists of 8,545,929 base pairs. This bacterium has very large, linear chromosomes. It also has a very high GC nucleotide content, comprising approximately 70–74% of its DNA. Mapping the S. griseus chromosomes is difficult due to their genetic instability. Streptomyces bacteria generally undergo DNA rearrangements, such as amplification and deletion, particularly at their ends. The S. griseus genome is currently being sequenced at the University of Tokyo **(Habib, 2016).**

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