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Molecular Identification of Local Isolated *Streptomyces* Species from North Region soil in Iraq

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Abstract

In this study *Streptomyces* were isolated from 50 bacterial isolates taken from 30 soil samples, these samples were collected from various locations in Iraq's various regions. The species of *Streptomyces* were isolated using starch casein agar and diagnosed microscopically and morphologically by Gram staining and glass slide. The sequence analysis 16S rRNA is used to report 11 *Streptomyces*. 10 bands of DNA gene, a result of specific polymerase chain reaction PCR, are elected from bacterial local isolates where 1000 base pairs within one volume, The PCR products of DNA samples were chosen from 11 local isolates based on nitrogenous base sequences. These organisms are revealed as a result of the study and by using DNA Blast NCBI as fellows; *Streptomyces gancidicus, S. werraensis, S. griseorubens, S. hawaiiensis, S. thermocarboxydus, S. cyaneus, S. misionensis, S. bellus, S. parvulus, S. labedae,*

Keywords: *Streptomyces*. specific PCR, Sequencing analysis

التشخيص الجزيئي لأنواع *Streptomyces* المعزولة محلياً من ترب المنطقة الشمالية في العراق

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الخلاصة

تم في هذه الدراسة ، الحصول على 50 عزلة بكتريا من جنس *Streptomyces* اخذت من 30 عينة تربة، جمعت من مواقع مختلفة من شمال العراق. عزلت العينات باستخدام اكار كازائين – النشأ وشخصت مايكروسكوبياً ومورفولوجياً بواسطة صبغة كرام والشريحة الزجاجية. تحليل التتابع 16 srRNA استخدم للكشف عن 11 عزلة من جنس *Streptomyces* تم اختيار 10 حزم من الـ DNA كنتيجة للتفاعل البوليمري المتسلسل المتخصص، انتخبت العزلات المحلية المعزولة عند 1000 قاعدة نيتروجينية ، تم كشف عن البكتريا قيد الاختبار باستخدام برنامج DNA Blast NCBI وكانت النتائج على النحو التالي: *Streptomyces gancidicus, S. werraensis, S. griseorubens, S. hawaiiensis, S. thermocarboxydus, S. cyaneus, S. misionensis, S. bellus, S. parvulus, S. labedae,*

الكلمات المفتاحية: *Streptomyces*- البوليمر المتسلسل المتخصص – تحليل المتتابع

Introduction

The species of *streptomyces* are widely prevalent in soil, It is the largest genus of the Actinomycetes, Gram-positive bacteria. Their colors mostly are grey but few are red, green, and white, while the blue is the rarest [1]. The genus *Streptomyces* is considered from Actinomycetes diameter (0.5-2) µm. It is also grown Aerial mycelium carrying many spores, that are arranged in chains and take different shapes, from the spiral form, Rectus- flexibilis form, Retinaculum- Apertum form [2] [3].

The *Streptomyces* genus is the most important species from the group of filaments. The Novel organisms can produce secondary metabolites (Antibiotics) and (Enzyme) production used to control many pathogenic bacteria and their inhibitory impact on harmful microbes [4] [5]. The higher rate of GC is ~70% of the content of *Streptomyces* spp. To separate *Streptomyces* from other bacterial such as Actinobacterial, there are distinguishing features such as 16S rDNA analysis and DNA-DNA hybridization [5] [6]. Geosmin, a scented substance produced by *Streptomyces* is responsible for the distinctive odor of soil.. This research aims to identify and isolate *Streptomyces* from the soil of northern Iraq (Nineveh, Duhok, Erbil) as well as diagnose them using microscopic and morphological experiments and a PCR test based on 16S rRNA to establish their genetic sequence. In addition, DNA Blast NCBI was used.

Materials and procedures

Collecting of Samples

Thirty soil samples were collected from various farm locations in Iraq's northern area, ranging in depth from 5 to 15 cm, and after being collected the samples were treated with calcium carbonate CaCO3 (1:10) and dried at 40-45 °C for four days. The samples were then placed in polyethylene bags and tightly sealed before being placed in the refrigerator until needed. (1 gm) was thoroughly mixed in tubes of 15 ml distilled water, followed by a series of dilutions until the sixth dilution was reached. On it (which was cooled to 45°C), the culture medium (starch-casein medium) was filtered. and 1ml of the last dilution was put in a sterile petri dish. This was done three times per sample. Several solitary colonies were used for re-culture in the same medium for pure culture after the plates were selected with (10-35) colonies [7] [8] [9].

Characteristics of *Streptomyces*

According to Bergy's manual of systematic bacteriology, second edition, the Actinobacteria, Part A [10] . The characteristics of *Streptomyces* are tasted based on the pattern of formation, Gram stain, and colony morphology.

Streptomyces detection

Based on the morphology and color of the colonies, the isolates were known As well as grow on Streptomyces as well as the Tryptone yeast extract glucose Agar, Glycerol Asparagine Agar, and Nutrient Agar. The aerial and medial twigs, as well as the arrangement of spores, were studied using the Slide culture technique [11].

The media

Starch-casein medium

This medium was made by combining: (10 gm) starch, (0.3 gm) casein, (2 gm) KNO3, (2 gm) NaCl, (0.02 gm) CaCO3, (2 gm) KH2PO4, (0.05 gm) MgSO4.7H2O, (0.01 gm) FeSO4.7H2O, (18 gm) Agar, in 1 liter of distilled water, at a pH of this medium was used in isolation [12].

Tryptone-yeast extract glucose agar

The agar was made by combining (10 gm) glucose, (3gm) yeast extract, (5 gm) tryptone, (1 gm) KH2PO4, (1 gm) K2HPO4, (20 gm) agar in 1 liter distilled water with (7.2) pH, and sterilizing it in an autoclave [13]. This was the medium that was used to make the diagnosis..

Glycerol asparagine agar medium

This medium was prepared by mixing: (1 gm) asparagine, (10 gm) glycerol, (1 gm) K_2HPO_4, (20 gm) agar, (1 ml) of trace salt solution, (0.64) gm $CuSO_4.5H_2O$, (0.11 gm) $FeSO_4.7H_2O$, (0.79 gm) $MnCl_2.4H_2O$, and (0.15) gm $ZnSO_4.7H_2O$, in (1 liter) of distilled water with 7.4 pH, The autoclave was used to sterilize items. This medium was used for diagnosis and isolation. [14]

Czapic Dox Agar_Dox agar medium

This medium was made by combining (30) gm sucrose, (3) gm NaNO3, (1) gm K2HPO4, (0.5 gm MgSO4, (0.5 gm KCl), (0.01) gm FeSO4, (15 gm agar) in 1 liter distilled water with a pH of 7.3, and sterilizing everything in the autoclave. [15]. This medium was used to isolate and identify the species.

Nutrient agar medium

This medium was made by melting (23 gm) of nutrient agar in (1 liter) of distilled water with a pH of 7.2 and sterilizing everything in the autoclave, as directed by the supplier company (Lab M Neogen Culture media). This medium was used in the isolation and identification of bacteria..

DNA from *Streptomyces* purification and acquisition

The DNA from the *Streptomyces* samples was extracted using Geneaid's kit analysis.

PCR Reactions

The Tri-EDTA (TE buffer) solution was used to fine-tune the DNA concentration in all of the isolated samples in order to achieve the optimal concentration for PCR reactions, and it worked well (50 nanogram per microliter). The master reaction for each PCR reaction was made by combining the DNA sample, the gene's specific primer, and the appender pre-mix in a (0.2ml) Eppendorf tube provided by the British company (bio). Using distilled water, the reaction volume was reduced to 20 microliters, and the components were then combined in a microfuge for (3-5) seconds. After that, the tubes were put in a thermal cycler to perform the polymer reactions, which were regulated by special software for each reaction. After that, the samples were electrophoresed in wells of a 2 percent agarose gel for 60-70 minutes. [3][4][16].

DNA Sequencing analysis

The most common and important method for detecting single nucleotide polymorphism (SNP) mutations and variations in DNA samples is DNA sequence analysis. On the other hand, the results of PCR reactions are used to determine the sequence of the amplified parts of DNA that will be used to find and study mut. DNA sequencing findings have recently been found to be extremely accurate in identifying mutations [5].

Furthermore, if the PCR reaction yielded more than one strand, it was purified and the desired fragment of DNA was extracted by the gel; however, if the reaction yielded just one strand, it was the main strand, and it was used to evaluate the sequences [17].

Using the method of DNA sequencing to determine the nucleotide sequences of the amplified section

The results of the PCR reaction for the samples mentioned earlier, as well as the primers, were read by a Hitachi 3130 Genetic Analyzer device, indicating that the samples used in the study are diagnosis. The genetic sequence data was linked to the National Center Biotechnology Information NCBI database., The BLAST software was used to examine the findings.

Results and Discussion

Thirty soil samples were collected from various locations in Iraq's northern region, and 50 *Streptomyces* samples were isolated. The samples were chosen based on the colonies' chalky appearance in the media and the wet soil (earthy odor) the smell of rain. [18][19]. The addition of CaCO3 to the soil at a ratio of 1:10 and a temperature of 40-45 0C has a significant impact drying the soil inhibits the growth of vegetative bacteria, and adding CaCO3 raises the pH, inhibiting the growth of fungi, enabling thread-like bacteria (*Streptomyces*) to thrive in the primary isolation [20][21][22].

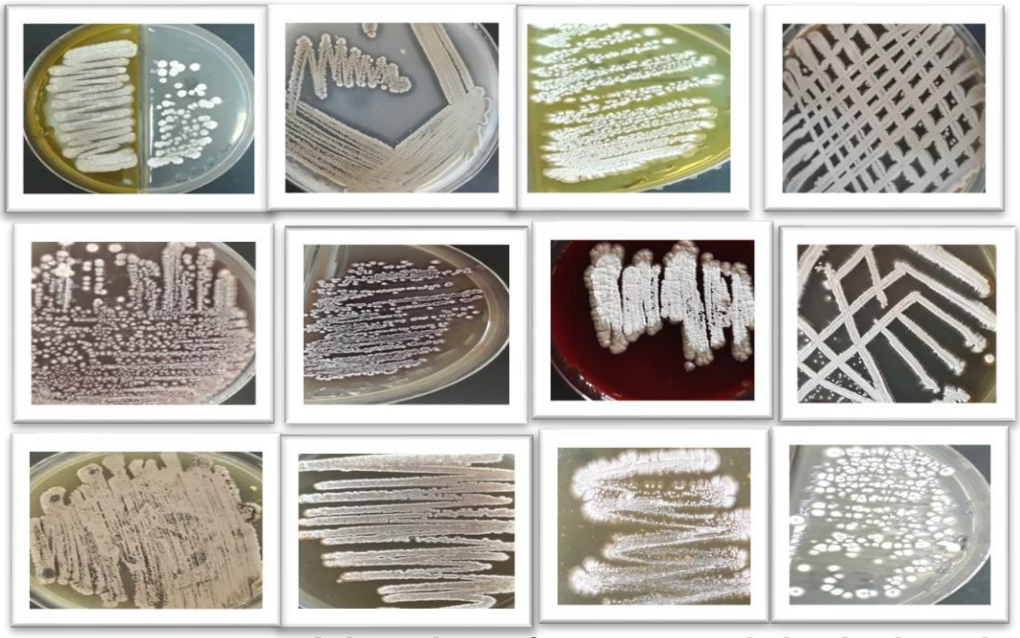

Figure 1: Morphology colonies of *Streptomyces* , which isolated aerial

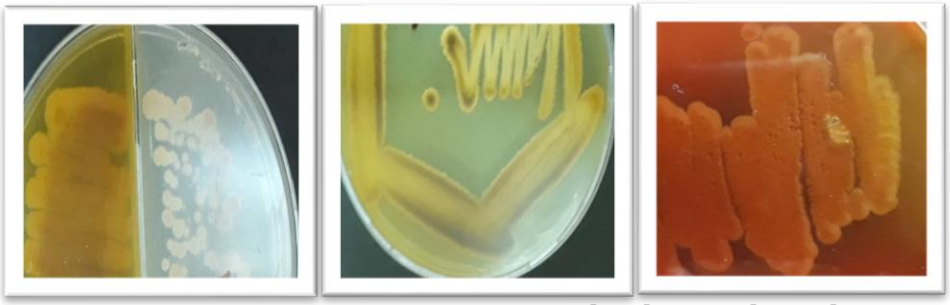

Figure 2: Streptomyces species isolated ground mycelia

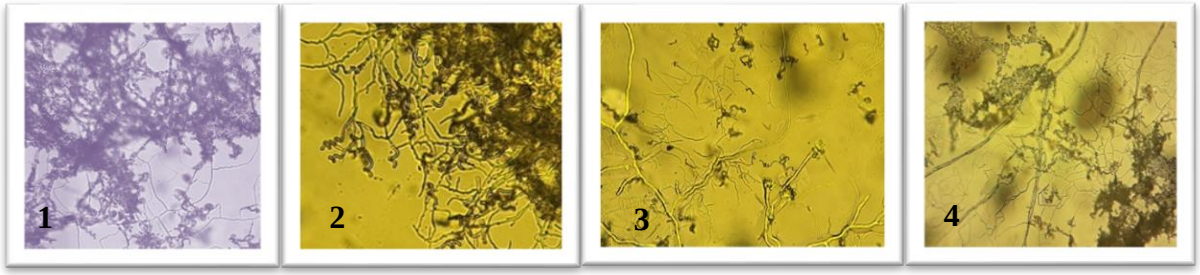

Figure 3:(1) Gram staining of isolated *streptomyces* under 100x magnification. (2,3,4): Glass slide technology

Diagnosis

The slide culturing method was used to diagnose *Streptomyces* samples, which is thought to be one of the best ways (on a genus level) to expose substrate and aerial hyphae, which are the distinguishing features that differentiate thread-like bacteria from one another. [8]

Strongly branched, unsegmented, and spore-free hyphae are present in the substrate. The threads of aerial hyphae are darker, thicker, and less branched than those of substrate hyphae. [3]. The sporophore, which can be erect (rectus), spiral (spiral), straight with a bent end (retinaculum-a cum), or straight with waves (rectus) (rectus-flexibilis), surrounds a long chain of spores in aerial hyphae [23]. When the isolates were cultured on different types of media, they displayed numerous colors, were incapable of forming melanin and other pigments, and the gray-colored colonies were the most visible (figure 1,2,3). These findings are close to those of [18].

Genomic DNA polymer reaction

Under the Geneaid procedure, a particular DNA reaction to Purified DNA was obtained from species collected from local samples.

Forward primer: [AAG CCC TGG AAA CGG GGT]

And the revers: [CGT GTG CAG CCC AAG ACA] [17][22]

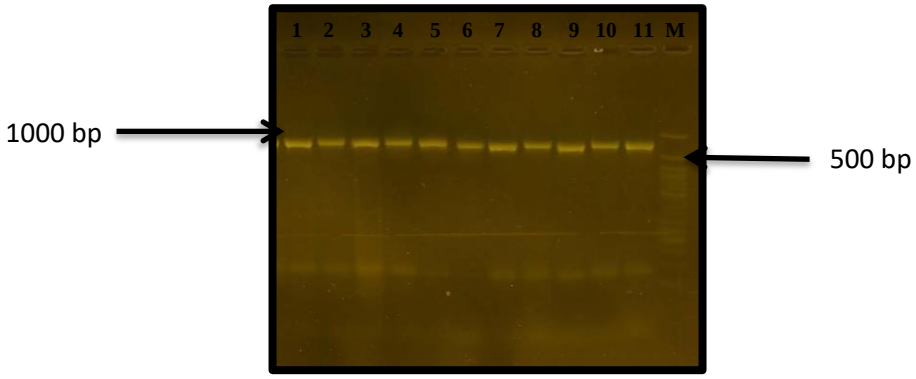

Fig.:4: **The results of the bacterial samples from the region' basic polymers reaction based on primer 16s rRNA (11) *Streptomyces* isolates**

In (Fig. 4) The samples with a similar length (1000 base) pairs generated from the *Streptomyces* reaction DNA-specific polymer show 11 strands of purified DNA. The presence of these strands demonstrates that these isolates' genomic DNA contains shared sequences of nitrogenous bases that can join with the primer and continue the reaction, resulting in new DNA strands of the same length. These findings are similar to those of [23]. As the sequences were obtained from the nitrogenous bases of the DNA samples as follows:

Sample MU1 (*Streptomyces gancidicus*)

GCCCCCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGGTAGC
CGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAG
GCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGG
ATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTG
CAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGT
TGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGGTTGTGAAAGCC
CGGGGCTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATC
GCAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCACGAGGACCCCGGTGGCGAACCCG
GATCTCTGGGCCGATACTG

These sequences were entered into a program DNA BLAST to show their types and how close they are to sequences in the Gene Bank, as the result of the analysis showed a similarity of (99%) between these sequences and the sequences of bacterial isolates registered in the Gene Bank with the number (MT588801.1) Fig. (5).

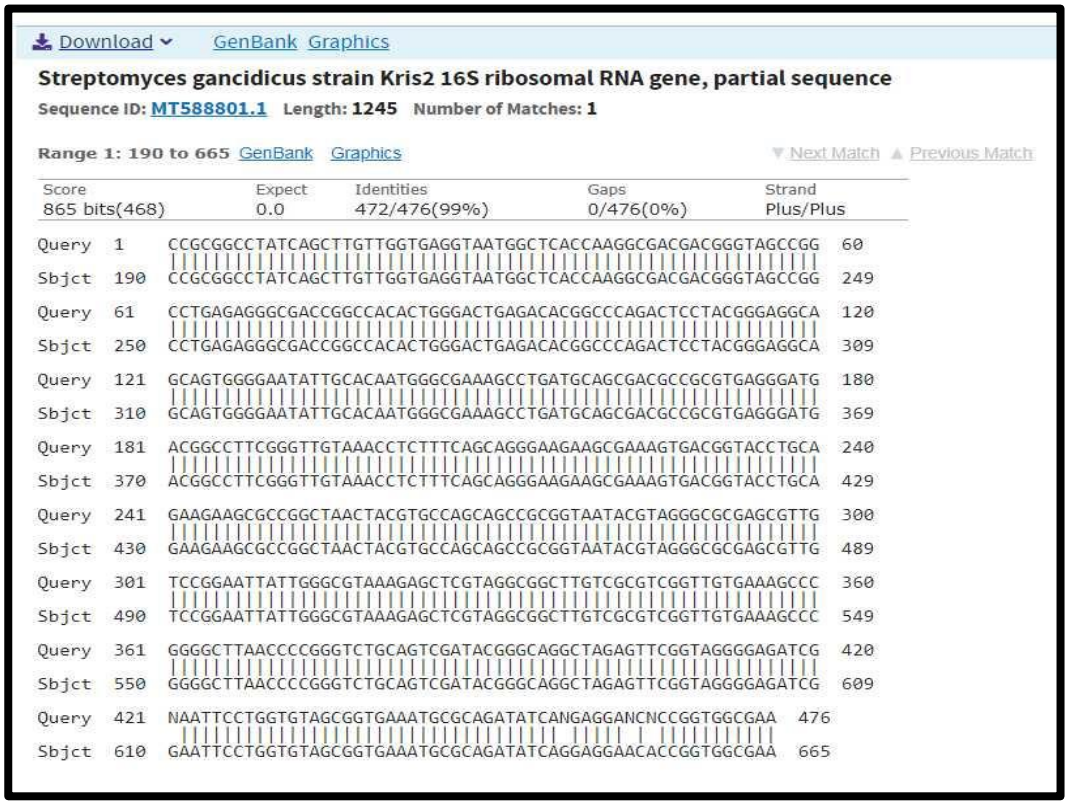

Figure: (5) Comparison of sequences of the nitrogen base between the local isolate (MU1) and standard strain (MT588801.1)

Sample MU2 (*Streptomyces werraensis*)

GGCGCACCCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGGT
AGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGG
GAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGA
GGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTA
CCTGCAGAAGAAGCGCCGGCTAACTCCCCGCCAGCAGCCGCGGTAATACGTAGGGCGCGA
GCGTTCCCCCGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGGTTGTGAA
AGCCCGGCCCTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAG
ATCGGAATTCCTGGTGTAGCGGTGAAATGCGCACATATCAGGAGGAACACCGGTGGCGAA
CGCGGATCTCTGGGCCGATACTGACGCTGAGGACCCAAAGCGTGGGGACCGAACAGGATC
ACATCCCCTGCCACCCCCCGCCGCAAACGGCGGGCACTACGTGTGGGCGACCTTCCCCCCC
CCCCCCCGCCCCCGCTACCCCCTTAACCGCCCCCCCTGGGGAGTACCGCCCCCCAGGC

The result of the program DNA BLAST analysis showed a similarity of (92%) between sequences of bacterial isolates registered in the Gene Bank with the number (MN179978.1) Fig. (6)

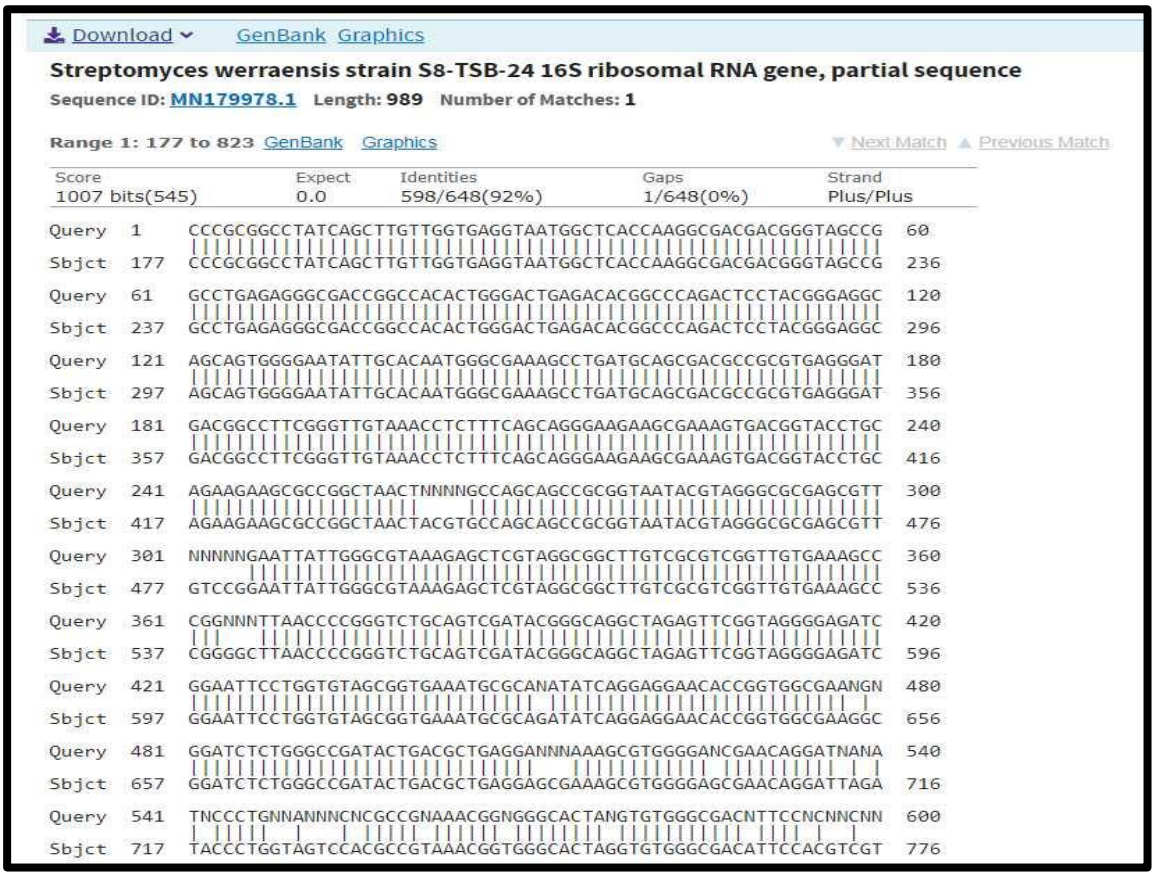

Figure: (6) Comparison of sequences the nitrogen base between the local isolate (MU2) and standard strain (MN179978.1)

Sample MU3 (*Streptomyces griseorubens*)

GTGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACG
GGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTA
CGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCG
TGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACG
GTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCG
CGAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGT
GAAAGCCCGGGGCTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGG
GGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGG
CGAAGGCGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAG
GATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCGACATTCCA
CGTCGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCT
AAAACTCAAAGGATTTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTAATTCGAC
GCAACGCGAAGAACCTTACCACGGCTTGACATACACCGGAAAGAGGCCCCCTTGCCGCCG
GCGACCGGCGCCCATCGGTCAGCTCCCGTCGCGAGCCGCTGGGTTAAGTCCGCAACCAGCG
CACCCTTG

The result of the program DNA BLAST analysis showed a similarity of (99%) between sequences of bacterial isolates registered in the Gene Bank with the number (MT525003.1) Fig. (7)

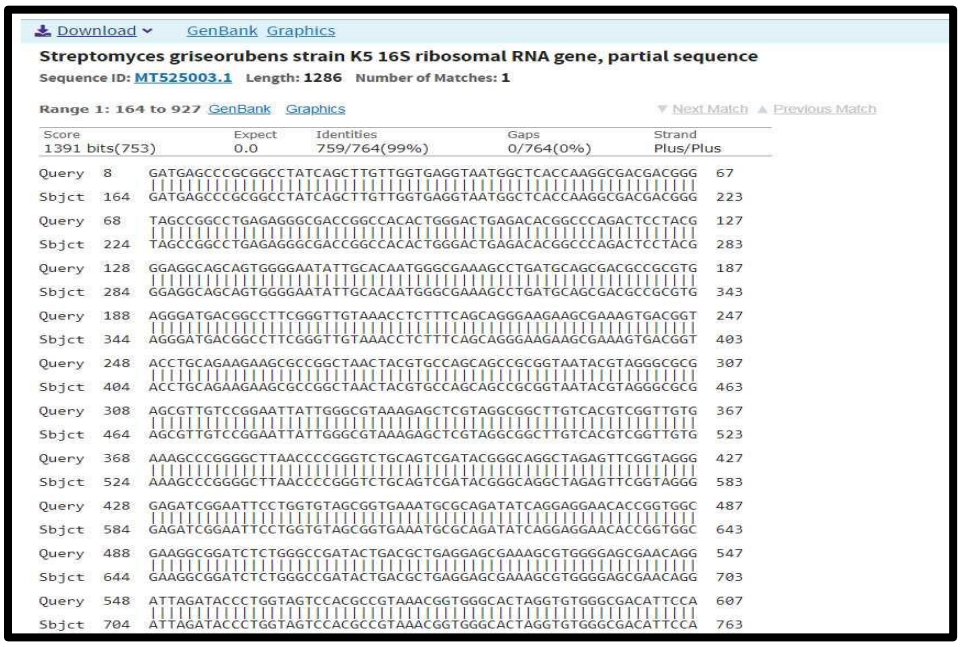

Figure: (7) Comparison of sequences the nitrogen base between the local isolate (MU3) and standard strain (MT525003.1)

Sample MU4 (*Streptomyces hawaiiensis*)

GGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAGTGGCTCACCAAGGC
GACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAG
ACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGA
CGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAA
AGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACG
TAGGGCGCGAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGT
CGGTTGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTC
GGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACAC
CGGTGGCGAAGGCGGATCTCTGGGCCGATAC

The result of the program DNA BLAST analysis showed a similarity of (91%) between sequences of bacterial isolates registered in the Gene Bank with the number (MT36169.1) Fig. (8)

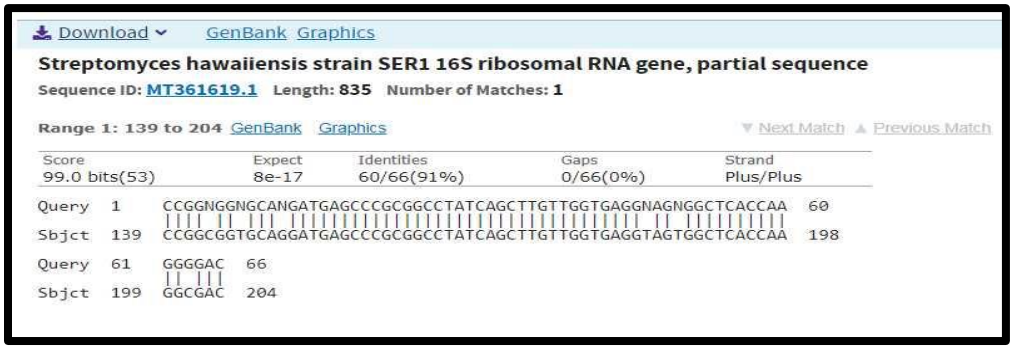

Figure: (8) Comparison of sequences the nitrogen base between the local isolate (MU4) and standard strain (MT36169.1)

Sample MU5 (*Streptomyces thermocarboxydus*)

CCCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGGTAGCCGG
CCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCA
GCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATG
ACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAG
AAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGCACGGCGCGAGCGTTGT
CCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCCCGTCGGTTGTGAAAGCCCGG
GGCTTAACCCCGGGTCTGCAGTCGATACGGCCAGGCTAGAGTTCGGTAGGGGAGATCGGAC
TTCCTGGTGTAGCGGCGAAATGCCCCCATATCACGAGGACCCCCCGGTGGCGAAAGCGGAT
CTCTGGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTACATAC
CCCTGGAGCCCCCCCGCCCACGGTGGGCCCTACGT

The result of the program DNA BLAST analysis showed a similarity of (96%) between sequences of bacterial isolates registered in the Gene Bank with the number (KU158245.1) Fig. (9)

Figure: (9) Comparison of sequences the nitrogen base between the local isolate (MU5) and standard strain (KU158245.1)

Sample MU6 (*Streptomyces cyaneus*)

CCTCCTTCGGGAGGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCA
CTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATACTGATCATCTTGGGCATCCTT
GGTGATCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGT
AATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGA
CTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGA
AAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAG
CAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGC
AGCCGCGGTAATACGTAGGGCGCGAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTA
GGCGGCTTGTCGCGTCGGTTGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCAGTCGATACG
GGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGA
TATCACGAGGAACACCGGTGGCGAAAGCGGATCTCTGGGCCGATACTGACGCTGAGGAGC
GAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGC
ACTAGGTGTGGGCGACATTCCACGTCGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCC
TGGGGGAGTACGGCCGCAAGGCTAAAACTCAAA

The result of the program DNA BLAST analysis showed a similarity of (99%) between sequences of bacterial isolates registered in the Gene Bank with the number (KM215731.1) Fig. (10)

Figure: (10) Comparison of sequences the nitrogen base between the local isolate (MU6) and standard strain (KM215731.1)

Sample MU7 (*Streptomyces misionensis*)

AGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGGTAGCC
GGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGG
CAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGA
TGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGC
AGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGTT
GTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCC
GGGGCTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCG
GAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCG
GATCTCTGGGCCGATACTGACGCTGAGGAGCGAAACCCTGGGGAGCGAACAGGATTACAT
ACCCTGGTACTCCACGCCGTACACGGTGGGCACTAGGTGTGGGCAACATTCCACGTTGTCC
GTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTCACACTCC
AAGGACTCGACCGGGGCCCCCACAAGCGGCGGACCATGCGGCTTACTCCCACCCCCCGCCA
AAAC

The result of the program DNA BLAST analysis showed a similarity of (97%) between sequences of bacterial isolates registered in the Gene Bank with the number (MT515826.1) Fig. (11)

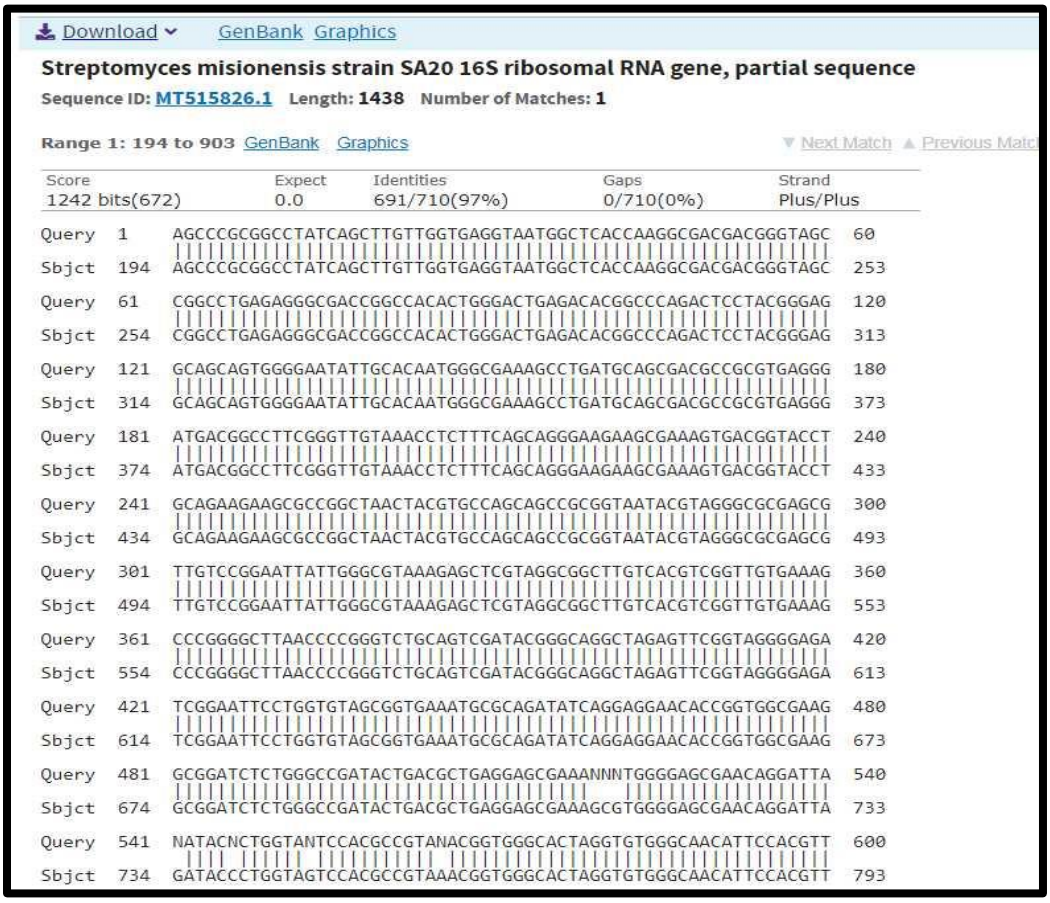

Figure: (11) Comparion of sequences the nitrogen base between the local isolate (MU7) and standard strain (MT515826.1

Sample MU8 (*Streptomyces bellus*)

AGTTTGATCATGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGAT
GAACCACTTCGGTGGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGC
ACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATACTGACCCGCTTGGGCATCCA
AGCGGTTCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGG
TAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGG
ACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCG
AAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCA
GCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAG
CAGCCGCGGTAATACGTAGGGCGCGAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGT
AGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCAGTCGATAC
GGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAG
ATATCAGGAGGAACACCGGTGGCGAAAGCGGATCTCTGGGCCGATACTGACGCTGAGGAG
CGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGG
CACTAGGTGTGGGCGACATTC

The result of the program DNA BLAST analysis showed a similarity of (99%) between sequences of bacterial isolates registered in the Gene Bank with the number (MT355856.1) Fig. (12)

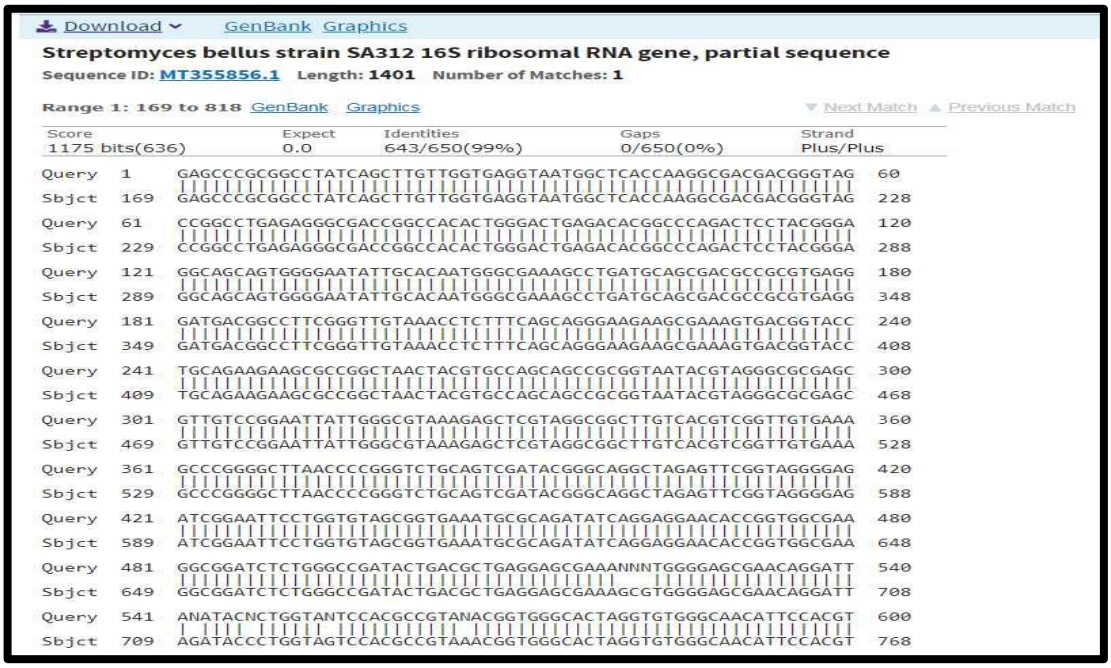

Figure: (12) Comparison of sequences the nitrogen base between the local isolate (MU8) and standard strain (MT515826.1)

Sample MU9 (*Streptomyces parvulus*)

GCGCCCCCCGCCTATCCCCTTGTTGGTGAGGCAATGGCTCACCAAGGCGACGACGGGTAGC
CGGCCTGAGAGGGCGACCGGCCACCCTGGGACTGAGACACGGCCCAGACTCCTACGGGAG
GCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGG

ATGACGGCCTTCGGGTTGTAAACCTCTTTCCCCACGGAAGAAGCGAAAGTGACGGCACCTG
CAGAAGAAGCGCCCCCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGT
TGCCCCCAATTATTGGGCGTAAAGAGCTCCTAGGCGGCTTGTCCCGTCGGCTGTGAAAGCC
CGGCGCTTACCCCCCCCCCCCCCCCCCCCCTACCCCCCCC

The result of the program DNA BLAST analysis showed a similarity of (93%) between sequences of bacterial isolates registered in the Gene Bank with the number (JX860396.1) Fig. (13)

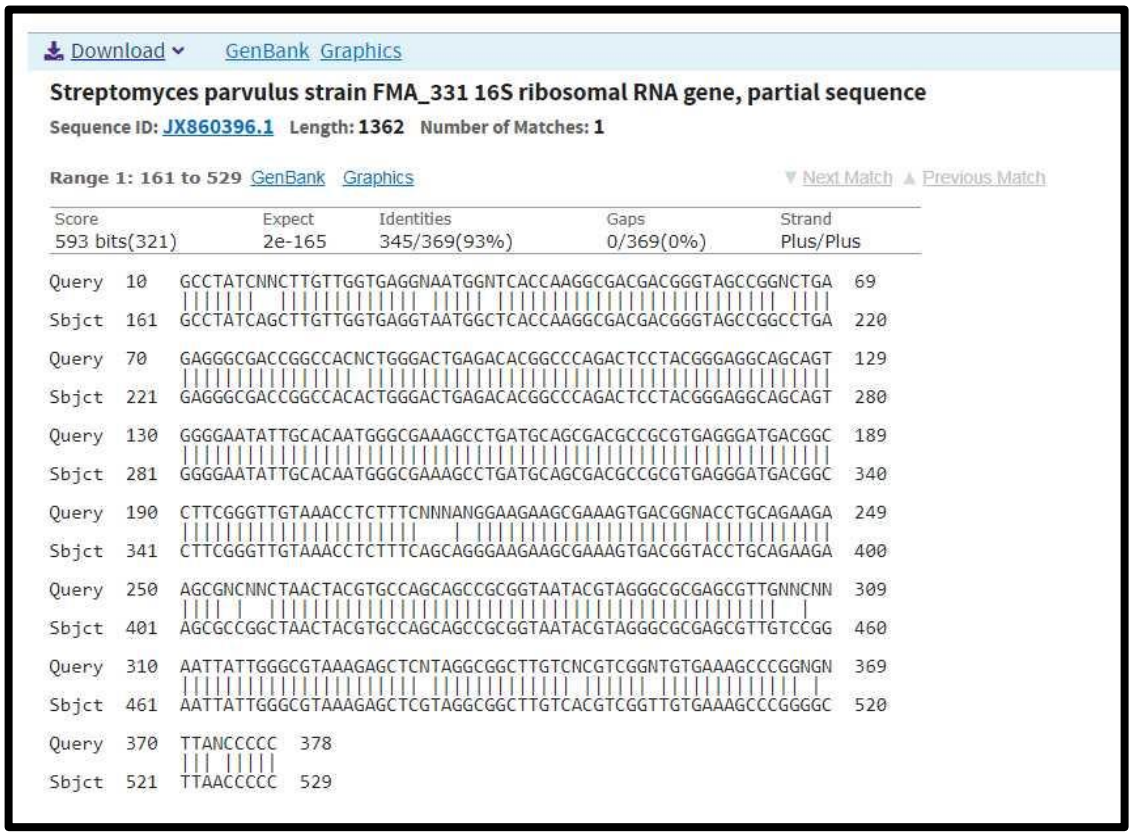

Figure: (13) Comparison of sequences the nitrogen base between the local isolate (MU9) and standard strain (JX860396.1)

Sample MU10 (*Streptomyces labedae*)

GAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGGTAGC
CGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAG
GCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGG
ATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTG
CAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGT
TGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCC
CGGGGCTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATC
GGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGC
GGATCTCTGGGCCGATACTGACGCTGACGAGCGAAAGCGTGGGGAGCGAACAGGATTAGA
TACCCTGGTAGTCCACGCCGCAAACGGTGGGCACTCCCGTGGGCGACCCCCCCCGTCGCCC

CGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTC
ACAGGAATTGACGGGGGCCCCGCCCCACCCGCCGGACCCTGTGGCTTATTCACGCACACTC
CGCT

The result of the program DNA BLAST analysis showed a similarity of (96%) between sequences of bacterial isolates registered in the Gene Bank with the number (JQ647891.1) Fig. (14)

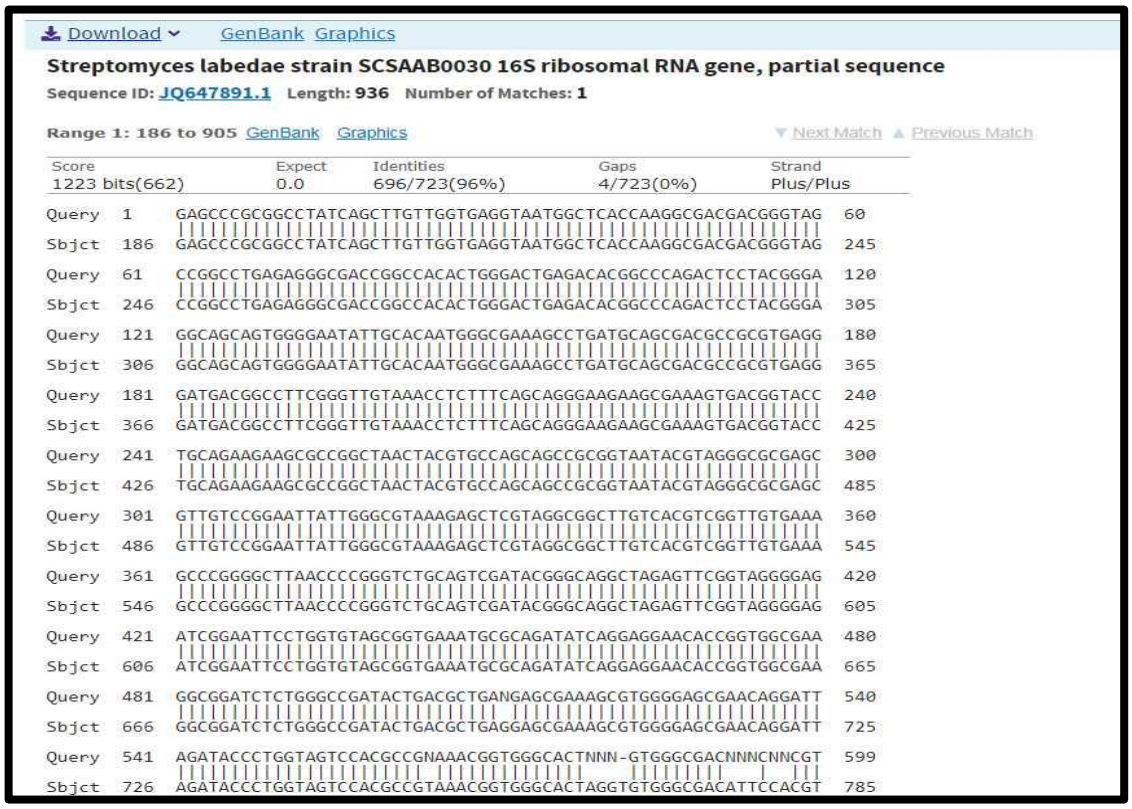

Figure: (14) Comparison of sequences the nitrogen base between the local isolate (MU10) and standard strain (JQ647891.1)

Sample MU11 (*Streptomyces variabilis*)

GGCGCACGACGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCCCCAAGGCGAC
GACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACT
CCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGC
CGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGT
GACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAG
GGCGCGAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGG
TTGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTACCAGTTCGG
TAGGGGAGATCGGAATTCCTGGTGTACCGGTGAAATGCGCAGATATCCCGAGGAACACCC
GGTGGCGAAGGCGGATCTCTGGGCCCGATACTGACGCTGAGGACCGAAAGCGTGGGGAGC
C

The result of the program DNA BLAST analysis showed a similarity of (97%) between sequences of bacterial isolates registered in the Gene Bank with the number (EU841660.1) Fig. (15)

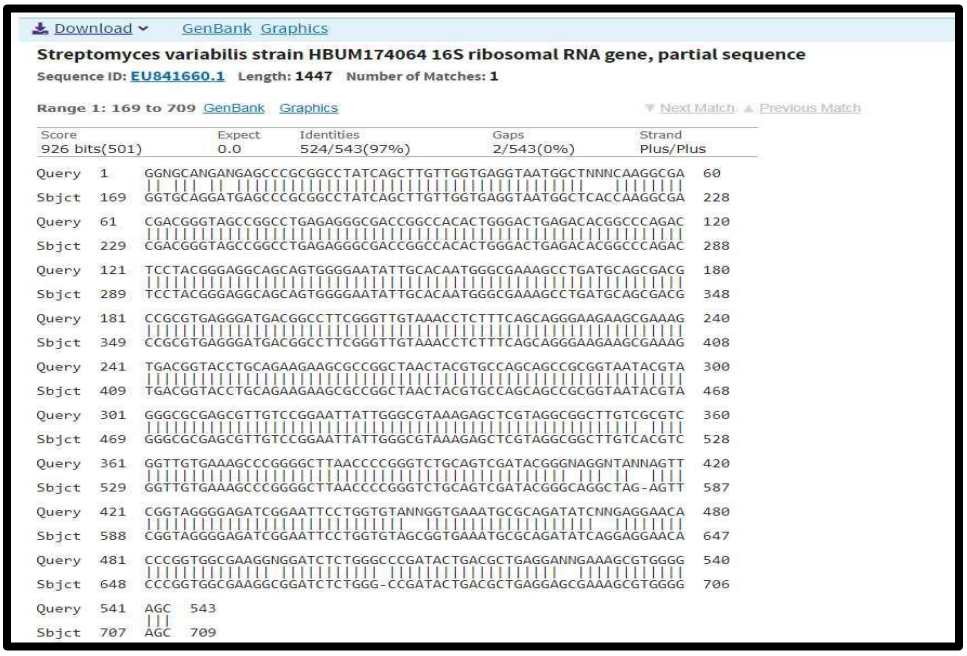

Figure: (15) Comparison of sequences the nitrogen base between the local isolate (MU11) and standard strain (EU841660.1)

The partial 16S rRNA sequences deposited in GenBank were analyzed using DNA Blast (Figure 5,6,7,8,9,10,11,12,13,14,15). It was discovered in that all isolates belong to a *Streptomyces* species with (77-99%) resemblance to the 16S rRNA series of closely related species. 16S rRNA, bacterial genetic maker often In chromosomal DNA, there is a multigene family or operons. The role of this gene has changed dramatically over time, and its size is now large enough for informatics purposes. [24].

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