MOLECULAR DETECTION OF NEW *STREPTOMYCES SPP.* FROM IRAQI OIL CONTAMINATED SOIL

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ABSTRACT

Streptomyces spp. **is one of Actinomycete that produce variety of secondary metabolite (antibacterial, antifungal and anticancer product). Bacteria that used in this study was isolated from oily soil in Baghdad /Iraq. The morphological characters was gram positive, branching, spore former. The goal of this research was to find genetic polymorphisms of one bacterial isolate (assigned S1) and to assess the accurate phylogenetic distribution of this isolated based on ribosomal sequences. In this work one genomic region was amplified that covered a portion of the 16S rRNA sequences. According to the identified results, the currently investigated sample was positioned within the major phylogenetic clade of the *Streptomyces albidoflavus* sequences in the generated comprehensive tree. In conclusion, the present tree provided an inclusive tool for the confirmed identity of the sample under investigation As a result, the PCR-sequencing approach was used in the studied sample has presented a confirmed identity of this sample and showed the pattern of its phylogenetic distribution. This sort of observed diversity may suggest the possibility to utilize the current 16S rRNA sequences in the accurate detection and discrimination for the *Streptomyces albidoflavus* sequences.**

Keywords: - Isolate, Antibacterial, Antiviral, genetic sequences

مجلة العلوم الزراعية العراقية -2023 :54(5):1298-1304 الزيدي وآخرون

التشخيص الجزيئي لجنس جديد من بكتريا ستربتومايسس من التربة العراقية الملوثة بالنفط

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المستخلص

بكتريا ستربتومايسز هي من البكتيريا الخيطية الاكتينومايسيتس إيجابية لصبغة جرام تنتج مجموعة متنوعة من مركبات الايض الثانوي (المضادات البكتيرية، المضادات الفطرية, ومضادات السرطان). تم عزل البكتريا المستخدمة في هذه الدراسة من التربة الزيتية في منطقة مصفى الدورة/ بغداد / العراق. وكانت الصفات الظاهرية والشكلية موجبة لملون جرام، متفرعة، ومكونة للبوغ. تم في هذه الدراسة التعرف على تعدد الأشكال الوراثية لعزلة بكتيرية واحدة (المسماه S1) ولتقييم التوزيع النشئي الدقيق لهذه العزلة بناءً على التسلسلات الريبوسومية. تم تضخيم موضع جيني يغطي جزءًا من تسلسل الرنا الريباسي S16 في هذه الدراسة. تم وضع العينة التي تم فحصها حاليًا داخل الفرع النشئي الرئيسي لتسلسل *Streptomyces albidoflavus* في الشجرة الشاملة التي تم إنشاؤها. كشفت شجرة النشوء والتطور أن هذه الاختلافات كانت مجرد انحراف طفيف داخل سلسلة *Streptomyces albidoflavus* الرئيسية، والتي تم دمج S1 فيها. استنادًا إلى التسلسلات الريبوزومية التي تم تحديدها، من خلال الدراسة نستنتج، قدمت الشجرة الحالية أداة شاملة للهوية المضمونة للعينة التي تم فحصها. لذلك، فإن استخدام استراتيجية تسلسل PCR في العينة التي تم تحليلها قد قدم هوية مؤكدة لهذه العينة وأظهر نمط توزيع النشوء والتطور. قد يشير هذا النوع من التنوع الملحوظ إلى إمكانية استخدام تسلسل الرنا الريباسي S16 الحالي في الكشف الدقيق والتمييز لتسلسل *Streptomyces albidoflavus*.

كلمات مفتاحية: عزلة، مضادات بكتيرية، مضادات فطرية، تسلسل جيني

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INTRODUCTION

Streptomyces *Spp*. bacteria are gram-positive filamentous isolated from the soil that have ability to produce a remarkable number of secondary metabolites (9) (1) and are characterized by a complex cycle of morphological distinctive treatment (4)(10). During the last few years, the Streptomyces genus and many other members of their DNA have been identified as high guanine and cytosine content (11) (8). Bacteria in the order Actinomycetales are common inhabitants of the soil with a unique ability to make antibiotics that are clinically useful (23),(20) (3). The initial observation of morphology was given to these organisms. InitiallyActinomycetes were once thought to be a cross between bacteria and fungi, but they are now considered prokaryotic organisms. Most Actinomycets population is one of the most important soil groups and are free living bacteria which are saprophytic in soil, water and colonized plants and are widespread in nature (12),(19).

MATERIAL AND METHODS

Soil collection: Soil samples were collected from contaminated soil with open borers (a depth of 100 cm and a diameter of 2,5 cm) at a depth of 60 cm, after which dried with air, thoroughly mixed with CaCO3 (10 percent w/w). Ten grams of sample of soil were mixed in a conical flask of 250 ml and one with 100 ml of sterilized distilled water (10^{-1}), agitated 10 min. By transferring 10 ml of aliquots to 4 flasks containing 90 ml of sterile distilled water (25). the last diluted (10^{-5}) was incubated with basal salt agar at 30°C for 10 days (13).

1. Gram staining method:

The stain has been prepared as per (15).

2. **Hydrophobicity test**

The differentiation in a complex media, consistent with the hydrophobicity of previously reported streptomycetes spores (5), was found to result in significant changes to the hydrophobicity of this organism (22),(18).

4. Extraction of Genomic DNA

The isolated samples' genomic DNA was extracted using the Genaid Kit according to the manufacturer's instructions. (Geneaid Biotech, Taiwan). A nanodrop was used to determine the concentration and purity of DNA. (BioDrop µLITE, BioDrop Co.,UK)(2).

5. PCR: PCR, or polymerase chain reaction, is a effective techniques for identifying various genes depending on their target sequences(16). For amplification, one PCR fragment was chosen., which respectively covered the 16S rRNA sequences, was amplified in this study (Tab.1). The forward sequence of the primer was 5- GATTAGTGGCGAACGGGTGA-3, and the reverse sequence of the primer was 5-CCTACGAGCTCTTTACGCCC-3. Bioneer was the source for the lyophilized primers. (Bioneer, Daejeon, South Korea).

RESULTS AND DISCUTION

Isolation of S1 bacterial isolate

This Bacteria **(***S1*) has been isolated from soil and have the code *S1 isolate*. Which was purification on basal salt agar.The observations showed that the strain of Iraq Streptomyces isolate was gram positively and rod-shaped. The microscopically characteristics were observed under 10x and oil-immersion (1000x) **(**Fig.1**).** The grammatical response of bacteria is an empirical examination, based on the marked differences in the structural ultra and chemical composition of the two primary forms in the form of prokaryotic cells. These two cell types are characterized by the existence or lack of an external lipid mucosal which, in the bacterial cells, is more reliable and fundamental (6). Both Gram-positive bacteria are limited by only one lipid membrane cell, and typically contain a large layer of peptidoglycan (20-80 nm) to maintain the gram-stain (6)**.**The Hydrophobic nature of Iraq *Streptomyces* isolate was given the positive result. This result due to the Iraq *streptomyces Spp.* which had the hydrophobic phenomena **(**Figure1**)**. The hydrophobicity of this organization changes dramatically when it is differentiated from starch casein agar, in line with the previously stated hydrophobic existence of streptomycetes spores (5,17).

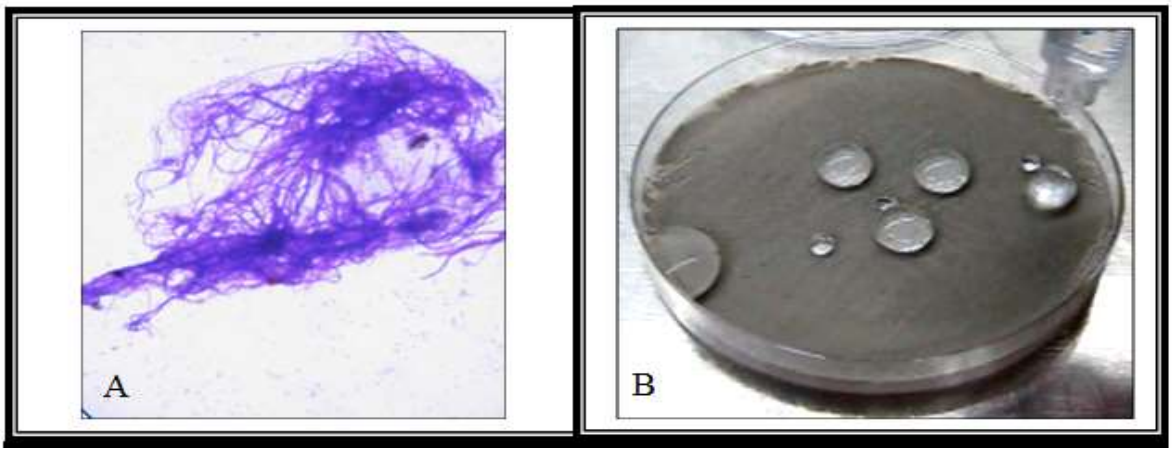

Figure1. The microscopically characteristics of *Iraqi Streptomyces isolate* were observed with Grams stain under 10x (A)and oil-immersion (1000x)(B) The Hydrophobic nature of *Iraqi Streptomyces isolate* on the Starch casein agar

Molecular characteristic

One sample was included in this locus, which revealed the exact lengths of the ribosomal segment. By using NCBI blastn to check the identity of the amplified products, the sequencing reactions revealed the amplified products' proven identification. The NCBI BLASTn engine showed a high sequence similarity between the samples that were sequenced and *Streptomyces albidoflavus* sequences (24). The NCBI BLASTn engine found roughly 99% percent homology with these predicted targets, which encompassed the requested regions of the rRNA gene sequences. The investigated sample showed a close homology with the GenBank accession number MT515831.1 that belonged to a Chinese strain of *Streptomyces albidoflavus* sequences. The exact locations and other features of the obtained PCR fragment were identified by comparing the observed DNA sequences of the currently analyzed sample with the retrieved DNA sequences as haven in. (Fig.2)(24).

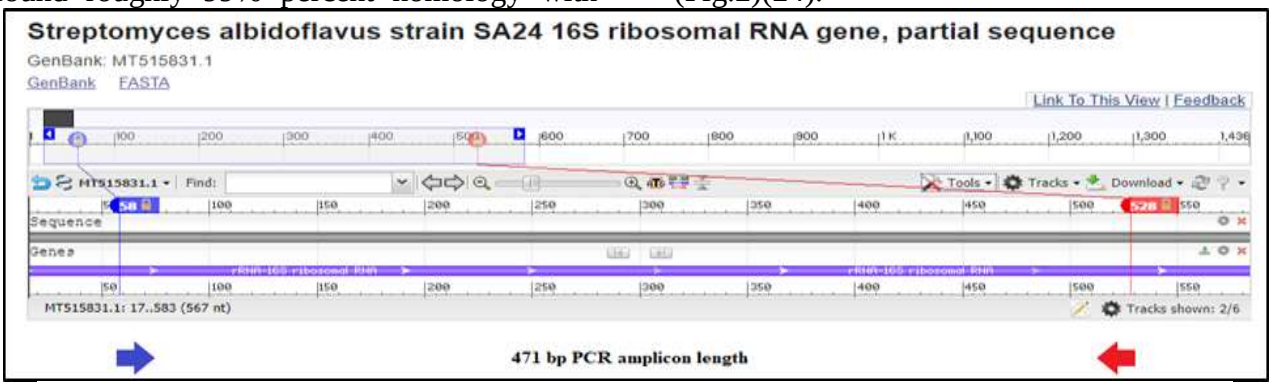

Figure 2 The exact position of the retrieved amplified fragments covered a portion of the 16S rRNA gene within the genomic sequences of *Streptomyces albidoflavus*. The blue arrow refers to the starting point of this amplicon while the red arrow refers to its endpoint.

Table 1. The positions and the amplified fragments length that were used to amplify a portion of the 16S rRNA gene within the bacterial genomic DNA sequences of *Streptomyces albidoflavus*.

Organism	Reference locus sequences (5′ - 3′)	length
Streptomyces albidoflavus	***GATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGACAAGCC CTGGAAACGGGGTCTAATACCGGATATGACCGTCTGCCGCATGGTGGATGGTGTAAAGCTC CGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAGTGGCTCACCAAGG CGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCA GACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCG ACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAA AGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGT AGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGG****	**471 bp**

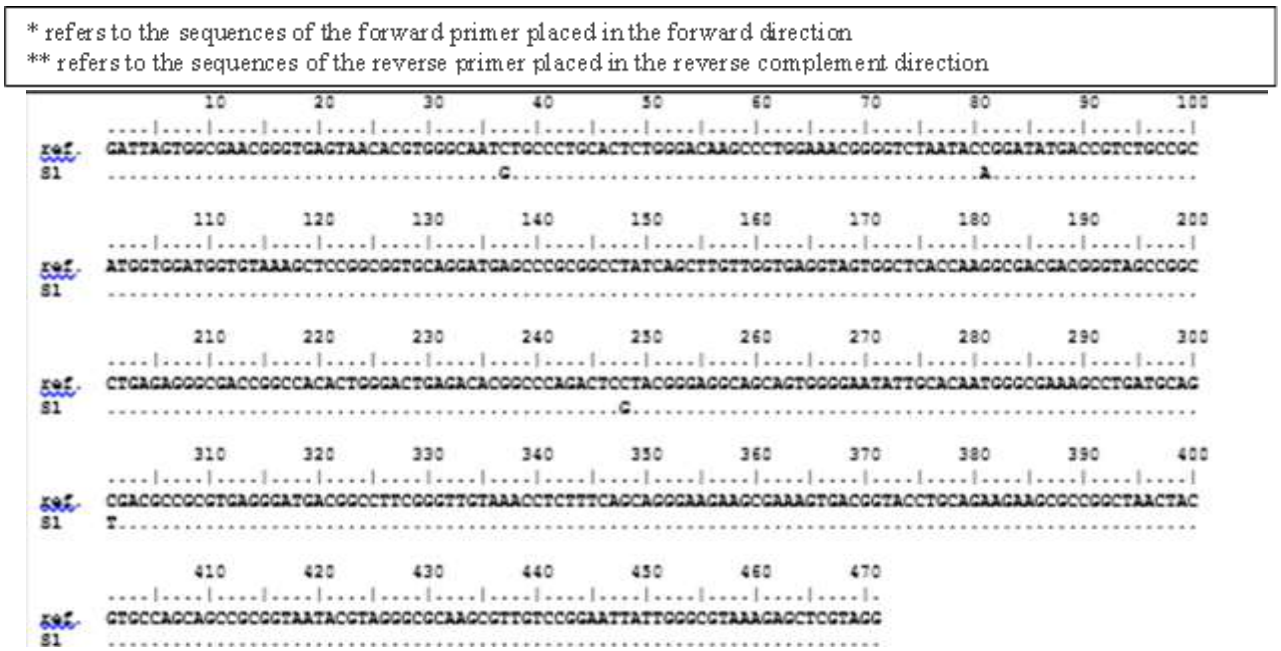

Figure 3. Nucleic acid sequence alignment of the studied sample with the most relevant deposited genomic sequences of *Streptomyces albidoflavus*The symbols "ref" and "S No.#" refer to NCBI reference sequences and sample numbers, respectively.

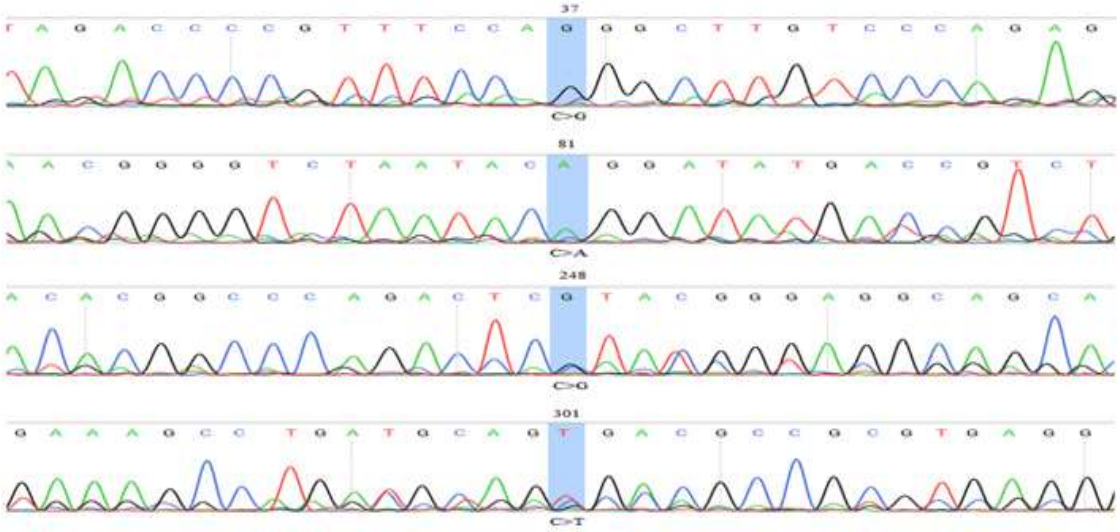

Figure 4. The chromatogram of the investigated bacterial sample of *Streptomyces albidoflavus*. The letter "S" refers to the code of the investigated samples in this study. the symbol ">" refers to substitution mutation.

The features of these sequences were highlighted inside the amplified sequences after situating the amplified segments within the rRNA sequences. (Tab.1). The recovered ribosomal sample sequences were compared to their matching reference sequences (Fig.3). These sequences were generated by aligning our research sample with the most closely related sequences in the NCBI database. (GenBank acc. no. MT515831.1). Three nucleic acid variations were observed in the currently investigated sample compared with the reference sequences. These observed variations were attributed to four nucleic acid substitutions (C>G 37, C>A 81, C>G 248, and C>T 301) (Figure. 4.(7). However, the analyzed sample's sequencing chromatograms, as well as its extensive annotations, were validated and documented, and the sequences' chromatograms were displayed in order of their positions in the PCR amplicons. The presence of these variants was confirmed in the original chromatogram. These detected nucleic acid substitutions were not found in the reference sequences of the *Streptomyces albidoflavus* genomic sequences (24). Based on the investigated 16S ribosomal nucleic acid sequences in the analyzed bacterial sample, a

thorough phylogenetic tree was produced. This phylogenetic tree includes the currently analyzed sample (S1) aligned with its highly related sequences in a neighbour-joining method, along with the other deposited DNA sequences. The total number of aligned nucleic acid sequences in the currently created tree was 100. The presence of only four species within the genus Streptomyces, which represents the tree's sole integrated nucleic acid sequences, was found in this comprehensive tree (14). These sequences were *Streptomyces albidoflavus, Streptomyces violascens, Streptomyces turgidiscabies,* and *Streptomyces misionensis*. Based on the analyzed genetic sequences, our 16S rRNA sequences were clustered into four major phylogenetic clades, which entailed a particular range of diversity of these bacterial sequences in terms of our analyzed rRNA sequences (Fig. 5) (21). One of these major clades was only represented by the *Streptomyces albidoflavus* clade, in which the currently investigated S1 sample was incorporated. However, this sample was positioned in the vicinity of the GenBank accession number MT515831.1, which was belonged to a Chinese strain of the *Streptomyces albidoflavus*. Furthermore, our sample was also suited in the vicinity to the GenBank acc. no. of MT515828.1, MT279915.1, .1, and MT131284.1 which were also belonged to other Chinese strains of the same organism. In a slight tilt to both mentioned strains, our sample was also positioned beside the GenBank acc. no. of CP040466.1 and CP040466.1, which were deposited from two Uruguayan strains of the same organism. Furthermore, the same data were also applicable to the other incorporated strains in the same clade (21). This sort of positioning referred to the presence of clear Asian – South American sources of our investigated sample. However, the currently observed variations (C>G 37, C>A 81, C>G 248, and C>T 301) were only a slight deviation within the main *Streptomyces albidoflavus* clade. Apart from this clade, three species of the same genus were also incorporated, namely *Streptomyces violascens, Streptomyces turgidiscabies,* and *Streptomyces misionensis*. It was found that the *Streptomyces violascens* clade resided in the vicinity of the *Streptomyces albidoflavus* clade. This observation showed the presence of a close phylogenetic association between both incorporated species. However, both *Streptomyces turgidiscabies* and *Streptomyces misionensis* clades were positioned in noticeable distant phylogenetic positions away from the *Streptomyces albidoflavus* clade. These large distances indicated the presence of distinct phylogenetic differences between the ribosomal sequences of *Streptomyces albidoflavus* species and *Streptomyces turgidiscabies,* and *Streptomyces misionensis*. These data indicated an obvious tendency of *Streptomyces albidoflavus* sequences to be incorporated away from these two species (14). From the above-stated data, it is consequent to consider two (Asian – South American) potential ancestries of the currently investigated sample. Thus, these suggested nationalities were revealed from the investigated 16S rRNA sequences of *Streptomyces albidoflavus*. These sorts of S1 genetic distribution referred to the sensitivity of the utilized rRNA ribosomal amplicons in the accurate discrimination among the investigated bacterial sample of *Streptomyces* sequences. As a result, the unique contribution of the constructed phylogenetic tree in the detection of the recently studied samples could not be ruled out. Accordingly, this notion provides a further indication of this analyzed *Streptomyces albidoflavus* isolate and reveals accurate genotyping phylogenetic distributions alongside highly relative sequences (21).

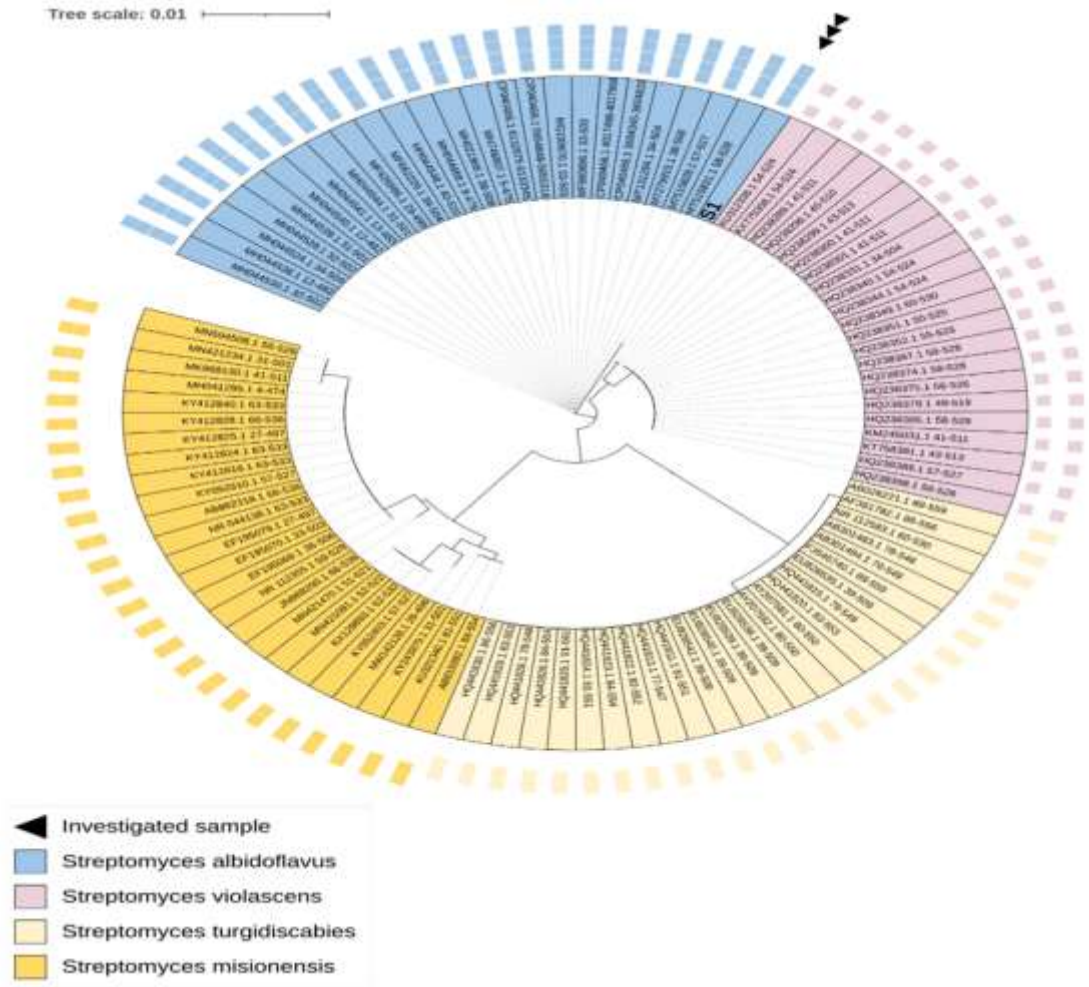

Fig. 5. The comprehensive phylogenetic tree of the 16S rRNA sequences within the genomic sequences of *Streptomyces albidoflavus* species. The variable colors reflect to how the investigated variations are grouped within their Genbank sequences. The number "0.01" in the upper left corner of the tree denotes the degree of scale range among the creatures classified by the comprehensive tree. The phylogenetic distances between the researched bacterial organisms are represented by the numbers in the treeThe letter "S" refers to the code of the investigated samples in this study

REFRENSES

1. Al-Abedi G.J.K. and Al-Amery M.A.M. molecular diagnosis and phylogenetic analysis of BABESIA spices isolated from ticks of infested cattle in Wasit governorate, Iraq. Iraqi Journal of Agricultural Sciences. 52(1):136-145. https://doi.org/10.36103/ijas.v52i1.1245

2. Ali, M., F. Abbas, and T. Mushtaq., 2021. Association of TGF-β2 gene polymorphism with Growth rate in local chickens. The Iraqi J. Vet. Med., 45(1): 9-16

3. Al-Obaidi R.M., Salih G.F. and Nore B.F.2021.bioactivity characterization of purified recombinant hypothetical protein coded by open reading FRAME-112 of STREPTOMYCES. Iraqi Journal of Agricultural Sciences. 52(2):502-511. https://doi.org/10.36103/ijas.v52i2.1314

4. Chater, K. F. 1993. Genetics of differentiation in Streptomyces.Annu Rev. Microbiol. 47: 685-713

5. Ensign, J. C. 1978. Formation, properties and germination of actinomycete spores. Annu Rev Microbiol32, 185-219

6. Gupta, R. S. 1999. What are Archaebacteria: Life's Third Domain or Monoderm Prokaryotes Related to Gram-positive Bacteria? A new Proposal for the Classification of Prokaryotic Organisms. Molecular Microbiology. 29(3):695-707

7. Hashim, H.O., M.K. Mohammed, M.J. Mousa, HH. Abdulameer, AT. Abdulameer, S.A. Hassan and M.B. Al-Shuhaib. 2020. Infection with different strains of SARS-CoV-2 in patients with COVID-19. Archives of Biological Sciences. 25:72(4):585

8. Hassan, S.A. and M.T.S. Al-Khateeb, 2017. Biological pre –treatment use local wild strain

of LIGNOLYTIC filamentous bacteria to improve in vitro dry matter digestibility and reduction lignin content of low quality roughages. Iraqi Journal of Agricultural science. 48: 6-11 (Special). https://doi.org/10.36103/ijas.v48iSpecial.2389. Horinouchi, S. and T. Beppu. 1990. Autoregulatory factors of secondary metabolism and morphogenesis in actinomycetes. Crit. Rev. Biotechnol. 10: 191-204

10. Hussein S.I., Aziz G.M., Shanshal R.M. and Ghani A.L. 2018. Determination the optimum conditions of lactase production from local isolate of STREPTOMYCES_SP. using sold state fermentation. Iraqi Journal of Agricultural Sciences. 49(4):685-693. https://doi.org/10.36103/ijas.v49i4.79

11. Khucharoenphaisan, K., N. Sripairoj and K. Sinma. 2012. Isolation and identification of actinomycetes from termite's gut against human pathogen. Asian J.Anim.Vet.Adv.,7:68-73

12. Kuster E. 1968. Taxonomy of soil Actinomycetes & Related Organisms. In : " Ecology of Soil Bacteria", eds. Gray (TRG) and Parkinson (D.), 322-336. Liverpool University Press, Liverpool

13. Lee, J.Y. and B.K. Hwang. 2002. Diversity of antifungal actinomycetes in various vegetative soils of Korea. Can. J. Microbiol. 48: 417

14. Letunic, I and P. Bork, 2019. Interactive tree of life (iTOL) v4: recentupdate s and new developments. Nucleic Acids Res. 2:47(W1): W256-W259.

15. Miles, R.S., J.G. Collee., B.P. Marion., A.G. Fraser., A. Simmons. and B. Watt. 1996. test for identification of bacteria in Mackie and McCartney Practical Medical Microbiology (14th ed.): Churchill-Livingstone; p: 133-150

16. Nadhom, B. N. 2018. Study of molecular composition of virulence bacteria isolated from bovine mastitis with biofilim production, Iraqi Journal of Agricultural Sciences . 49(5):840-846. https://doi.org/10.36103/ijas.v49i5.44

17. Nascimento, T. P., Sales, A. E., Porto, C. S., Brandão, R. M. P., Takaki, G. M. C., Teixeira, J. A. C., ... & Porto, A. L. F. .2015. Production and characterization of new fibrinolytic protease from Mucor subtillissimus UCP 1262 in solid-state fermentation. Advances in Enzyme Research, 3(03), 81-91

18. Niladevi b, K. N. and P. Prema, 2008. Immobilization of laccase from Streptomyces psammoticus and its application in phenol removal using packed bed reactor. World Journal of Microbiology and Biotechnology. 24:1215-1222. Iraqi J. of Agricultural Sciences –8201:49(4):685-693

19. Niladevi, K.N., R.K. Sukumaran, and P. Prema, 2007. Utilization of rice straw for laccase production by Streptomyces Psammoticus in solid-state fermentation. Indian Journal of Microbiology and Biotechnology. 34 (10):665-74. Iraqi J. of Agricultural Sciences –8201:49(4):685-693

20. Rakesh, K.N., N., Dileep, S. Junide, and T. R. Prashith Kekuda, 2014. Optimization of culture conditions for production of antimicrobial metabolites by bioactive Streptomyces species SRDP-TK-07. An international Journal of Advances in Pharmaceutical science. 5 (1): 1809 -1816. The Iraqi J. of Agricultural Sciences –8201:49(4):685-693

21. Sievers, F. and D. G. Higgins. 2014. "Clustal Omega, accurate Alignment of Very Large Numbers of Sequences." In Multiple Sequence alignment Methods, pp. 105-116. Humana Press, Totowa, N.J.

22. Smit, G., M. H. Sraver, B. Lugtenberg, and J. W. Kijne. 1992. Flocculance of *Saccharomyces cerevisiae* cells induced by nutrient limitation, with cell surface hydrophobicity as a major determinant. Appl. Environ. Microbiol.58**:** 3709-3714

23. William, F. and R.J. Paul. 2006. Developing a new resource for drug discovery: Marine actinomycete bacteria. Nat. Chem. Biol., 2: 666-673

24. Zhang Z, S. Schwartz, L. Wagner and W. Miller. 2000. A greedy algorithm for aligning DNA sequences. J. Comput. Biol. 7(1-2):203-14.

25. Zheng, Z., W. Zeng, Y. Huang, Z .Yang, J. Li, H. Cai and W. Su. 2000. Detection of antitumor and antimicrobial activities in marine organism associated actinomycetes isolated from the Taiwan Strait, China. FEMS Microbiol. Lett. 188:87-9 1.