Research Article

Phenotypic and Genotypic Characterization of *Streptomyces qinglanensis* Isolated from Local Soil Samples

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Abstract | A bacterial strain was isolated and characterized from the soil of the Tigris River. Morphological characteristics and biochemical tests revealed that the bacterium affiliate to the Streptomyces spp., due to the nature of white colonies, filamentous in appearance, and positive Gram's stain. This characterization was supported by 16S rRNA gene sequencing, confirming the isolate as belonging to the *Streptomyces qinglanensis* species with a 96% sequence identity. Growth experiments revealed that the isolate was able to growth at temperatures ranging from 15°C to 35°C, while it showed no growth at 4°C, 40°C, and 45°C, indicating that the organism is mesophilic and sensitive to both low and high temperatures. It also exposed a clear ability to tolerate high levels of salinity of up to 15% NaCl, reflecting its physiological adaptation to a moderately saline river environment. This adaptation is due to the presence of Na^+/H^+ pumps, as well as the presence of specialized membrane lipids such as iso-C15:0 and antheso-C17:0. Functionally, *Streptomyces qinglanensis* bacteria exhibits a clear enzymatic capacity to break down various organic compounds including cellulose, starch, and Tween 20, 40, and 80, through the production of active enzymes such as cellulase, amylase, catalase, and urease. These findings highlight the isolate's biological and physiological effectiveness, promising it for possible use in environmental and industrial applications, especially in the organic pollutant remediation and bio-enzyme production. In contrast, the isolate shows its inability to produce gelatinase, indicating the absence of this precise enzyme property.

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Introduction

Streptomyces spp. was first identified by (Waksman and Henrici, 1943). This genus is recognized as one of the most diverse of actinobacteria genera, containing more than 717 familiar species with documented scientific names (Law *et al*., 2018). This genus is known to be a renowned source of antibiotics

and other bioactive compounds, making it a significant resource in biotechnology (Alam *et al*., 2022).

Streptomyces species is primarily isolated from the soil. Still, isolates have also been isolated in the muddy soil of mangroves depending on centrifugation and differential separation techniques. Morphological detection of these isolates was achieved using light microscopy and scanning electron microscopy, after they were grown on ISP3 medium (International Streptomyces Project Medium No. 3) and incubated at 28°C for 15 days (Nisha *et al*., 2025). After two weeks of incubation, the culture appearances were evaluated according to the guideline established by Shirling (Shirling and Gottlieb, 1966).

As for the identification of Streptomyces colony colors, this was done based on the standard color charts developed by the joint ISCC (International Color Council) and NBS (National Bureau of Standards) committee (Antido and Climacosa, 2022). *Streptomyces qinglanensis* is a recently identified species within the genus Streptomyces, which is known as the most diverse genera of Actinobacteria in the environment. This genus is notable by its capacity to produce a broad spectrum of secondary bioactive compounds, particularly antibiotics. This bacterial type was first isolated from tropical forest soil on Hainan Island, China, specifically from the Qinglan area of Wenchang County, and its name reflects the region of its origin (Law *et al*., 2017). The genus Streptomyces, belonging to Streptomycetaceae family and is considered one of the extensively studied genera due to its industrial and medical relevance. These bacteria are recognized for their capacity to develop fungal-like hyphae and generate aerial spores, giving their colonies a unique appearance resembling mushrooms, even though they are bacteria. It also plays a crucial role in soil fertility and biodynamics due to its ability to decomposing of soil organic matter (Hopwood, 2007) .

Streptomyces qinglanensis is identified as a Gram-positive bacterium and is characterized by a high nuclear content of guanine-cytosine (GC), a defining feature of this genus. It has been shown that this species shares numerous chemical and physiological characteristics with its related species, but genome and proteomic analyses have uncovered subtle variations in genetic makeup and cellular composition, supporting its classification as a new and independent species (Xu *et al*., 2012).

This species holds great potential source for the discovery of new secondary compounds, such as antibiotics and antifungal agents and antitumor compounds. Given the growing global challenges related to antibiotic resistance, investigating new Streptomyces species is a priority for the development of effective natural-origin drugs (Lacey and Rutledge, 2022). Molecular analyses based on 16S rRNA gene sequencing demonstrate that *S. qinglanensis* forms a distinct evolutionary lineage within the Streptomyces genus. Chemotaxonomic studies also identified that this species produces a unique combination of sugars, fatty acids, and cellular polymers, which are which serve as taxonomic markers used to distinguish species within the Actinobacteria (Barka *et al*., 2016). Ecologically, *S. qinglanensis* contributes a major role in carbon and nitrogen cycling through the decomposition of organic matter in tropical ecosystems. Its ability to produce substances that suppress the growth of other microorganisms is also believed to help preserve the balance of the terrestrial microbiome and enhance plant resistance to various diseases (Ramírez-Elías *et al*., 2014: Alwan *et al*., 2025; Talib *et al*., 2025). The study of *S. qinglanensis* significantly enhances the genetic and applied understanding of the genus Streptomyces, opening new avenues for discovering unique metabolic pathways and novel chemical structures that could contribute to the development of innovative pharmaceutical and agricultural products. worldwide research efforts continue to analyze this species focusing on whole genome sequencing, biochemical characterization, and bioactivity assays of this species (Barka *et al*., 2016; Lacey and Rutledge, 2022).

Materials and Methods

Soil samples were collected from the banks of the Tigris River in Amarah, Maysan Governorate, southern Iraq, to isolate filamentous bacteria of the genus Streptomyces. Ten-fold serial dilutions of the samples were prepared using nutrient broth and incubated at 28°C for 24 hours, following incubation time, the dilutions were plated onto plates of ISP4 medium and incubated at the same temperature for 7 days. Once bacterial colonies appeared, these colonies were purified several stages of purification using slant streaking and re-cultivation method to obtain pure isolates (Shirling and Gottlieb, 1966).

Morphological and biochemical examinations

Morphological identification was conducted using Gram staining, where morphology and cellular characteristics were observed under a light microscope to detect whether the bacteria were Gram-positive or Gram-negative. The isolates' ability to grow at different temperatures was also tested: 4°C, 15°C, 28°C, 35°C, 40°C, and 45°C. The isolates grown in plates were incubated for 7 days, and phenotypic growth was evaluated at each temperature (Feng *et al.*, 2023). The isolates' growth was also evaluated under salt stress conditions, where a tolerance test was conducted to different concentrations of sodium chloride (NaCl) in the culture medium using the following concentrations: 0%, 1%, 5%, 7%, 9%, 10%, and 15%. The samples were incubated at 28°C for 7 days. In addition, the ability of the isolates to utilize a number of compounds as carbon or energy sources was examined. These compounds include sodium citrate, cellobioses, arabinose, starch, gelatin, urea, as well as compounds (20, 40, and Tween 80). Standard methods were used in these tests as stated in the taxonomic manuals for actinomycetes (Jiao *et al.*, 2024).

Genetic characterization and phylogenetic tree

From pure single colony of morphologically and biochemically identified Streptomyces species, DNA was extracted and purified depending on Qiagen company's instructions. Polymerase chain reaction was used to magnify 1450 bp segment of the 16S rRNA gene by a universal 16S rRNA primers 27F-5'AGAGTTTGATCCTGGCTCAG3', 1492R-5'-GGTTACCTTGTTACGACTT-3' (Miyoshi *et al.*, 2005) 2µl of pure DNA (50 ng/l), 3µl from each forward and reverse primers (62.5 mol/l), 25 µl Bioneer master mix and deionized H2O were added to achieve the total volume of 50 µl. 3Prime thermocycler programmed with the following temperature profile; a gene was used to incubate reaction through 96 °C as initial denaturation step for 3 min., followed by 27 cycles of amplification, denaturation at 96 °C for 30 s, primers annealing at 56 °C for 25 sec., primer extension was at 72 °C for 15 sec. and finally at 72 °C for 10 min. (Miyoshi *et al.*, 2005). PCR amplification result was analyzed using 1.0 % agarose gel and DNA marker (100 bp) using 1X TBE buffer for 40 minutes at 120mA and 65V, ethidium bromide was used as DNA stain. GBOX F3 computerized UV transilluminator was used to observe the amplified nucleic acid. NCBI web site was employed to assess the similarity between the PCR products and the existing gene sequences via data base queries (National Centre for Biotechnology Information, 2020).

A neighbor joining phylogenetic tree was created to analyze the relationships of the obtained sequence. The BLAST tool provided a list of closely related sequences from numerous Streptomyces species and other related taxa. Characteristic sequences with high percent identity were selected for comparative analysis. These sequences were used to generate a phylogenetic tree, illustrating the phylogenetic relationship between the isolate and relevant species, according to sequence similarity identified via BLAST.

Results and Discussion

Bacterial isolates were obtained from the soil of the Tigris River banks in Amara city/Maysan Governorate/southern Iraq. The morphological and biochemical characteristics of the bacterial isolates were studied on ISP4 medium, where they appeared as white colonies and were purified several times to obtain single pure isolates. After that, the isolates were stained with Gram stain, and the tests showed that the bacterial isolate was Gram positive and thread-like in shape with a purple color under the electron microscope, as shown in Figure 1 and Figure 2. These results were consistent with (Emthomya *et al.*, 2025) who have confirmed that *Streptomyces qinglanenses* bacteria were Gram positive with white colonies in culture dishes.

Figure 1: *Color of S. qinglanensis on ISP4 medium*

Figure 2: *Morphology and pigmentation of S. qinglanensis observed under a light*

The results shown in Table 1 revealed that the bacterial isolate grew at temperatures of 15, 20, 28, and 35°C, but no growth was observed at 40 and 45°C. This may be due to the damage of proteins and enzymes caused by high temperatures, especially enzymes that begin structural decomposition at temperatures above 37°C, which leads to the cessation of vital processes and growth (Lertcanawanichakul and Sahabuddeen, 2023).The other reason is due to the disruption of the function of the cell membrane, as high temperatures disrupt the function of the cytoplasmic membrane and lead to the loss of ionic and water balance, which is necessary for the continuity of cellular life (Prescott *et al*., 2005). Growth conditions also showed no growth at 4°C, as this is considered a very low temperature and unsuitable for their biological or enzymatic activity. At this temperature, most mesothermal bacteria enter a dormant state and do not grow, because enzymes lose their effectiveness at this cold temperature and metabolic rate decreases significantly (Xu *et al*., 2012). The results of the salt concentration tolerance test (NaCl) showed that the bacteria grew in a range of (15, 10, 9, 7, 5, 1, 0), which indicates that the bacteria tolerate salinity. The reason is due to the environment from which they were isolated, which is the soil of the banks of the Tigris River, which had a salinity ratio of 2500 TDS, which made them adapt themselves physiologically to withstand the osmotic pressures resulting from salts. This result was consistent with what was stated by (Dmitrieva *et al*., 2025) who showed that *Streptomyces qinglanenses* bacteria grow in salt concentrations of NaCl from 0% to 25%. He also noted that the bacteria contain fatty acids like as iso-C15:0 and anteiso-C17:0, which contribute the membrane flexibility and stability under saline conditions. According to the report of (Sleator and Hill, 2002), these bacteria possess Na^+ / H^+ antiporters that assist remove excess sodium from the cell and reduce the toxicity of incoming salts.

Table 1: *Biochemical tests for Streptomyces qinglanenases*

Test result	Test
+	Cellulose degradation
+	Starch hydrolysis
+	Catalyse production
+	Urease production
-	Gelatin hydrolysis
+	Tween 20,40,80 hydrolysis
	temperature growth
+	4°C
+	15°C
+	20°C
+	28°C
+	35°C
-	40°C
-	45°C
	Growth in salt concentrations (NaCl)
+	0
+	1
+	5
+	7
+	9
+	10
+	15

The results also demonstrated that *Streptomyces qinglanensis* has the ability to degrade multiple organic compounds, such as cellulose and starch. It also produces important bio-enzymes such as catalase and urease, in addition to degrading 20, 40, and Tween 80. This is due to the advanced physiological and enzymatic characteristics associated with the Streptomyces genus. The bacteria produce the enzyme cellulase, which breaks down cellulose into simple sugars such as glucose. This is common in Streptomyces for its role in decomposing plant material in the soil (Rathnan and Mechoor, 2011). Starch degradation is carried out by the enzyme amylase, which decomposes starch into maltose and glucose. Streptomyces is a known source of amylase production (Datta, 2024). Catalase production, which decomposes hydrogen peroxide into water and oxygen, protecting the cell from oxidative stress (Xu *et al*., 2012). Urease production, which decomposes urea into ammonia and CO_2, allows bacteria to use urea as a nitrogen source (Zhou *et al*., 2025). Regarding

the degradation of Tween 20, 40, and 80, the results indicated that *Streptomyces qinglanensis* is capable of degrading these compounds due to its ability to produce lipase and esterase enzymes that break down fats in its environment (Hu *et al*., 2012). As for the gelatin degradation test, the result was negative, due to the fact that the bacteria do not produce the gelatinase enzyme.

Analysis of DNA sequences

Amplified PCR fragments of targeted gene were sent to the MACROGEN company sequencing analysis. Using Geneious Prime 2019 software version 1.1. The raw sequences were visually reviewed and edited. The partial sequence of 844 base pairs was analyzed by searching the NCBI BLAST tool to determine its similarity with known sequences in the database. The sequence showed high similarity to *Streptomyces qinglanensis*, with the accession number MF682454.1, confirming the identity of the isolate at the species level.

The successful amplification of the 16S rRNA gene segment using universal primers and the subsequent sequencing allowed for accurate molecular identification of the previously morphological and biochemical characterized *Streptomyces* species. The 16S rRNA gene is a highly conserved genetic marker widely used for bacterial taxonomy and phylogenetic studies, making it a reliable choice for species-level identification (Srinivasan *et al*., 2015). The partial sequence obtained (844 bp) revealed a strong match with *Streptomyces qinglanensis* (accession no. MF682454.1), it obviously that the isolate belongs to this species. This finding is aligned with the morphological and biochemical characteristics observed, supporting the accuracy of the initial identification process. According to study of (Miyoshi *et al*., 2005),the optimized thermal cycling parameters facilitated specific amplification of the target gene segment. The electrophoretic analysis vitrified the presence of a well-defined PCR product of the expected size, which is essential to prevent sequencing errors due to unintended amplification. Staining with ethidium bromide and visualization under UV light demonstrate confirm clear evidence of successful PCR amplification. The identification of *Streptomyces qinglanensis* enhance our understanding of the genetic diversity and distribution of Streptomyces species, a genus notable for their secondary metabolite production and biotechnological potential.

A neighbor-joining phylogenetic tree was generated to clarify the evolutionary relationship of the obtained sequence (Query-2015121) with Streptomyces species. By employing the BLAST tool, multiple closely related sequences were identified, including *Streptomyces smyrnaeus*, *Streptomyces qinglanensis*, Streptomyces taxa, all indicating high sequence similarity to the query (Figure 3).

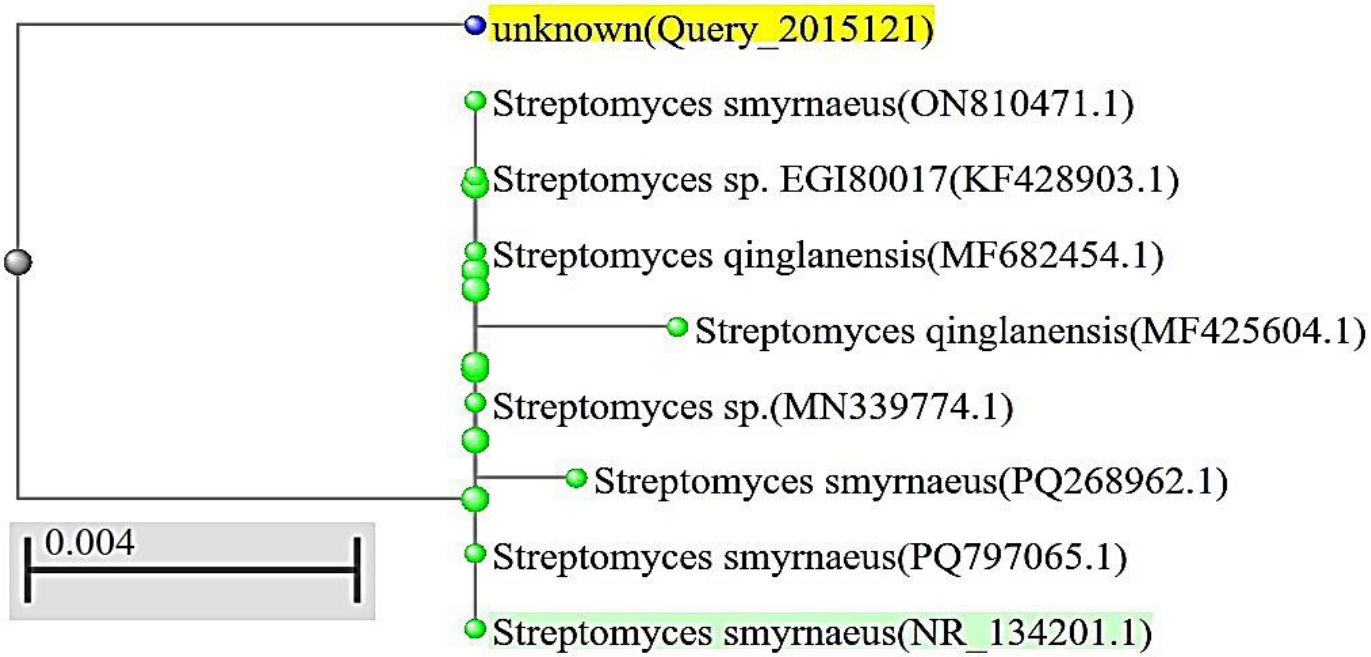

Figure 3: *Neighbor-joining phylogenetic tree showing the relationship between the query isolate and closely related Streptomyces species based on 16S rRNA gene sequences. Branch lengths represent genetic distances.*

In the constructed phylogenetic tree, the unknown isolate clusters tightly with *Streptomyces qinglanensis* (MF682454.1 and MF425604.1) and *Streptomyces smyrnaeus* strains, indicating a close genetic affiliation (Figure 3).

The creating of the neighbor-joining tree not only confirms the isolate's identity but also enhance our understanding of its evolutionary relationships within the Streptomyces genus. This information is important for future studies exploring the genetic diversity, ecological roles, and potential biotechnological applications of Streptomyces species.

Conclusions

The uses of both conventional microbiological techniques with molecular methods such as PCR amplification and sequence analysis constitutes a powerful approach for the accurate identification of bacterial isolates. The identifying of *Streptomyces qinglanensis* in this study paves the way for further investigations into its potential benefits.

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Novelty statement

This study presents the important detailed phenotypic and genotypic characterization of *Streptomyces qinglanensis* isolated from local soil samples, expanding the understanding of its diversity and ecological distribution.

Author's Contributions

Abdulridha Ati Jaafar: Conceived and designed the study; drafted the manuscript.
Fadhil Neamah Al-Kanany and Mustafa Adnan Idan: Performed the experiments and collected the data; drafted the manuscript.
Mohammed Sabeeh Majeed: Analyzed and interpreted the data; All authors reviewed and approved the final version of the manuscript for submission.

Generative AI and AI-assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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