RESEARCH ARTICLE

Molecular detection of *Streptomyces* antibiotic production genes *StrA* and *StrB*

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Received: April 16, 2024; **accepted:** June 10, 2024.

Streptomyces*, a genus of Gram-positive bacteria known for their filamentous morphology and remarkable ability to produce a diverse array of antibiotics, play a pivotal role in combating infectious diseases. *Streptomyces* can grow in various environments and are similar in shape to filamentous fungi. This study aimed to isolate and characterize *Streptomyces* isolates from Hillah city, Iraq with a focus on identifying those harboring the *StrA* and *StrB* genes, which are crucial for streptomycin production, and evaluate their antibacterial activity against pathogenic bacteria. A total of 100 samples were collected from different regions and locations at Hillah city. Only 25 of them were diagnosed with *Streptomyces and* grown and purified more than one time on International *Streptomyces* Project-2 (ISP-2) agar medium. All these samples were tested for their antibacterial activity against pathogenic bacteria of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Klebsiella pneumonia*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The morphological characteristics of aerial hyphae and mycelium of *Streptomyces* spp. were identified by using a light microscope at 1,000X on ISP-2 agar medium. The results showed that 8 of 25 *Streptomyces* isolates had antibacterial activity against pathogenic bacteria through secondary screening by agar wells diffusion method. The genes responsible for antibiotics production in the *Streptomyces* isolates (*StrA* and *StrB*) showed their antibacterial ability against pathogenic bacteria at (J-C-93) isolate that encoded by *StrA* gene, and (J-C-93, M-S-29) isolates that encoded by *StrB* gene. By characterizing *Streptomyces* isolates from Hilla city, Iraq and identifying those possessing the *StrA* and *StrB* genes associated with streptomycin production, the study provided a basis for discovering novel antibiotic candidates. Furthermore, the findings expanded our understanding of *Streptomyces* diversity in a specific geographic region, which is crucial for both drug discovery efforts and ecological studies of these important bacteria.

Keywords: *Streptomyces*; *StrA*; *StrB*; molecular detection; antibiotic production.

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Introduction

The fight against infectious diseases is a continuous battle throughout history, dating back to the ancient Egyptians using moldy bread to treat wounds. The discovery of penicillin by Alexander Fleming in 1928 marked a turning point in this ongoing struggle [1]. Today, antibiotics remain essential in modern medicine, saving countless lives and revolutionizing

healthcare. Among the unsung heroes of this battle are bacteria belonging to the genus *Streptomyces*. These filamentous, Gram-positive bacteria, often mistaken for fungi due to their morphology, are prolific producers of a diverse array of antibiotics [2]. *Streptomyces* thrive in various environments worldwide, including soil, decaying matter, and even marine environments [2]. Their remarkable adaptability and metabolic versatility allow them to synthesize a vast array of secondary metabolites, many of which possess potent antimicrobial properties [3], and associate with the specific genes such as *StrA* and *StrB* for this crucial function [4].

Streptomyces are easily distinguished from other bacteria by their unique filamentous morphology, resembling that of fungi [5]. They form a network of branching hyphae called mycelium. Aerial hyphae extend from the substrate mycelium, producing spores that allow *Streptomyces* to disperse to new environments. These bacteria are ubiquitous in the environment, demonstrating remarkable adaptability to diverse ecological niches [6]. They are primarily found in soil, where they play a crucial role in decomposing organic matter and cycling nutrients. *Streptomyces* have also been documented in marine sediments, freshwater environments, and even within the gut of insects and other animals [7]. This wide ecological distribution underscores their remarkable versatility and resilience. The genus *Streptomyces* encompasses thousands of species, resulting in a vast array of secondary metabolites with diverse biological activities. Of particular note, many of these metabolites are antibiotics with the potential to combat a wide range of pathogenic bacteria. *Streptomyces* are renowned for their ability to produce a wide variety of antibiotics, often referred to as secondary metabolites [7, 8]. These antibiotics are synthesized through complex biosynthetic pathways encoded within their genomes, involving the coordinated action of numerous enzymes [8]. Antibiotic production in *Streptomyces* is often triggered by environmental cues such as competition with other microbes. This "chemical warfare" strategy provides *Streptomyces* with a competitive advantage in their ecological niche by inhibiting the growth of neighboring bacteria. Understanding the genes responsible for antibiotic production in *Streptomyces* is crucial for harnessing their full potential in the fight against infectious diseases [6, 9]. The *StrA* and *StrB* genes are part of larger gene clusters responsible for the biosynthesis of specific antibiotics in *Streptomyces*. These genes encode enzymes that catalyze crucial steps in the assembly of antibiotic molecules. While the precise functions of *StrA* and *StrB* may vary depending on the specific antibiotic, their presence often indicates the potential of a *Streptomyces* isolate to synthesize that particular antibiotic [7, 10]. By identifying isolates harboring these genes, their potential to produce antibiotics with activity against pathogenic bacteria can be predicted.

The exploration of *Streptomyces* in the local area can provide valuable insights into the untapped potential of this region's microbial diversity. The isolation and identification of *Streptomyces* isolates with antibacterial activity suggest the presence of novel antibiotic candidates. Further investigation of these isolates, including characterizing the antibiotics they produce and evaluating their efficacy against a broader range of pathogens, could lead to the discovery of new tools in the fight against infectious diseases. This study aimed to isolate and characterize *Streptomyces* isolates from various locations in Hilla city, Babylon, Iraq, screen these isolates for the presence of the antibiotic production genes (*StrA* and *StrB*), and evaluate the antibacterial activity of the isolates against a panel of pathogenic bacteria. The findings of this study would contribute to the understanding of the untapped potential of *Streptomyces* diversity in Hilla city and their role in addressing the global challenge of antimicrobial resistance.

Materials and methods

Sample collection

Soil samples were collected from various locations within Hilla city, Babylon Governorate, in the center region of Iraq between February 2022 and August 2023. The geographic coordinates of the sampling sites were 32.474693/44.426633, 32.505750/44.408872, 32.524305/44.431830, 32.494259/44.466232, 32.474491/44.453425, 32.443049/44.425631, and 32.394168/44.399744. At each sampling site, soil samples were collected at a depth of 10-15 cm using sterile trowels and placed into sterile plastic bags. The soil type at each site was classified as an area suspected to be contaminated with *Streptomyces* genera. The soil types collected included sandy loam, clay loam, and organic-rich soils, reflecting the diverse soil types found in Hillah city. To minimize contamination by other bacteria and fungi, calcium carbonate was added to the soil samples upon arrival at the laboratory [7].

Preparation the International *Streptomyces* Project-2 (ISP-2) agar medium

The media for the growth of *Streptomyces* were prepared according to the method of Rajeswari *et al* [8]. ISP-2 agar is a rich growth medium that is commonly used for cultivating various *Streptomyces* species, including *Streptomyces lydicus* and other plant root-associated strains. ISP-2 medium was prepared by mixing 4 g yeast extract, 10 g malt extract, 4 g dextrose, 20 g agar with distilled water up to 1 L volume and autoclaved at 121°C for 15 minutes.

Isolation *Streptomyces* spp. samples from soil

Each soil sample was sieved separately to remove gravel, stones, debris, plant residues, and pollutants before incubated at 55°C for 5 minutes to reduce contamination. The samples were then diluted using normal saline to create a series of dilutions ranging from 10^{-1} to 10^{-6}. The dilutions were then plated on ISP-2 agar medium and incubated at 28°C for 5-7 days in a Binder CB-60 incubator (BINDER GmbH, Mittleren Ösch, Tuttlingen, Germany) [9, 10].

Screening of the antimicrobial activity

A glass beaker with a capacity of 500 mL was used to grow *Streptomyces* bacteria. Each beaker contained 250 mL of ISP-2 broth medium and incubated for 7 days at a temperature of 28°C. After the incubation, the cellular components were separated by using Whatman No. 1 filter paper (Battlefield Enterprise Park, Shrewsbury, United Kingdom). The cell-free supernatant was obtained after centrifuging the sample at 6,000 rpm for 15 minutes using a refrigerated Micro-220 centrifuge (Hettich, Iphofen, Germany). An equal volume of ethyl acetate was then added to the cell-free supernatant and vigorously shaken until the two layers (aqueous and organic) separated. The aqueous layer was discarded, and the alcohol layer containing the metabolites was evaporated and dried using a Buchi Rotavapor R-210 (Buchi Labortechnik AG, Flawil, Switzerland) to obtain the secondary metabolites for further analysis. The antibacterial susceptibility of the metabolites produced by *Streptomyces* was tested using the diffusion well method against a group of pathogenic bacteria obtained from the advanced microbiology laboratories at the University of Babylon, College of Science (Hillah, Babylon, Iraq). The extracted metabolites were kept at -18°C [13].

Morphological identification of *Streptomyces* spp.

All isolates were identified based on their morphological characteristics including colony appearance, aerial hyphae, and substrate mycelium. Gram staining was applied to examine the isolates under a Stemi 508 light microscope (Carl Zeiss Microscopy, LLC., White Plains, NY, USA) at 1,000X magnification.

Molecular analysis

Genomic DNA was extracted from *Streptomyces* isolates using the Favorgen Bacterial Genomic DNA Extraction Kit (Favorgen, Ping Tung, TAIWAN) following the manufacturer's instructions. Polymerase chain reaction (PCR) amplification of the *StrA* and *StrB* genes was performed using the primers of strA-F (5'- GCT CAA AGG TCG AGG TGT GG -3') and strA-R (5'- CCA GTT CTC TTC GGC GTT AG -3'), and strB-F (5'-

Table 1. Cultural characteristics of *Streptomyces* spp. isolates.

Streptomyces spp. Symbol	Aerial mycelia	Substrate mycelia
k-S-7	Grey white	Brownish grey
N-S-25	Grey white	Yellowish brown
M-S-29	White	Yellow
M-S-48	Greenish White	Yellow
H-S-66	Blue yellow	Yellow
H-S-75	Golden	Pale yellow
MA-S-90	White	Pale yellow
J-C-93	Pink white	Purple

GAC TCC TGC AAT CGT CAA GG -3') and strB-R (5'-GCA ATG CGT CTA GGA TCG AG -3'). PCR reactions were conducted in a Biometra TOne Series thermal cycler (Analytik, Göttingen, Germany) using a reaction mixture containing 1 µL of template DNA, 1 µL of each forward and reverse primers, 12.5 µL of Favorgen master mix, and 9.5 µL of nuclease-free water for a total volume of 25 µL. The PCR program consisted of 94°C for 5 minutes followed by 35 cycles of 94°C for 20 seconds, 59°C for 20 seconds, and 72°C for 30 seconds. A final extension step was performed at 72°C for 5 minutes. The PCR products were analyzed by electrophoresis on a 1.5% agarose gel alongside a 100-2,000 bp DNA ladder (Favorgen, Ping Tung, TAIWAN). PCR products of the expected size were purified and sequenced to confirm the identity of the amplified genes. Sequencing results were edited, aligned, and analyzed against the respective sequences in the reference database using Bio Edit Sequence Alignment Editor Software Version 7.1 (DNASTAR, Madison, WI, USA). Observed variations in each sequenced sample were numbered in the PCR amplicons, and their corresponding positions within the reference genome were noted.

Results and discussion

Morphological characteristic of *Streptomyces*

The *Streptomyces* colonies identified in this study were listed in Table 1. The isolates exhibited a range of aerial and substrate mycelia morphology when cultured on ISP-2 medium, as well as diverse colony colors (Figure 1). ISP-2 medium is known for its rich content of vitamins, amino acids, and nitrogen, along with dextrose, an important carbon source for bacterial growth [16]. One of the key advantages of ISP-2 over other ISP media such as ISP-3, ISP-4, and ISP-5 is its ability to clearly differentiate the colors of the aerial mycelium and its substrate, facilitating the identification of different *Streptomyces* species [17]. Therefore, ISP-2 is the preferred medium for studying the morphological characteristics of *Streptomyces* colonies, as it provides a reliable and accurate method for distinguishing between species. Using a light microscope at 1,000X magnification, eight *Streptomyces* isolates were identified to clarify the color and form of their mycelium and substrate. The colony colors observed in the Petri dishes after two weeks of incubation were diverse, including oriental, gray, brown, purple, and yellow. These findings aligned with previous studies that observed a correlation between the color of streptomycin-producing *Streptomyces* isolates and their specific strain type [18]. The colony color of the isolates in this study further supported this general trend.

Screening of the antimicrobial agent

The results of antibacterial screening test for *Streptomyces* cultures extracts against a panel of pathogenic Gram-positive, Gram-negative bacteria found that these extracts of isolates exhibited antibacterial activity (Figure 2). The results showed that 8 out of 25 *Streptomyces* isolates exhibited antibacterial activity. The

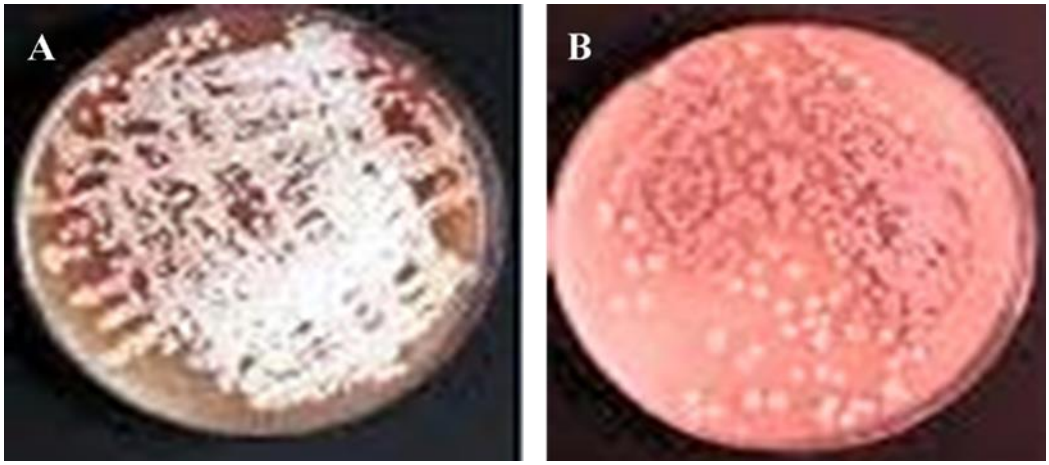

Figure 1. Cultural properties of *Streptomyces.* **A.** Top view. **B.** Bottom view.

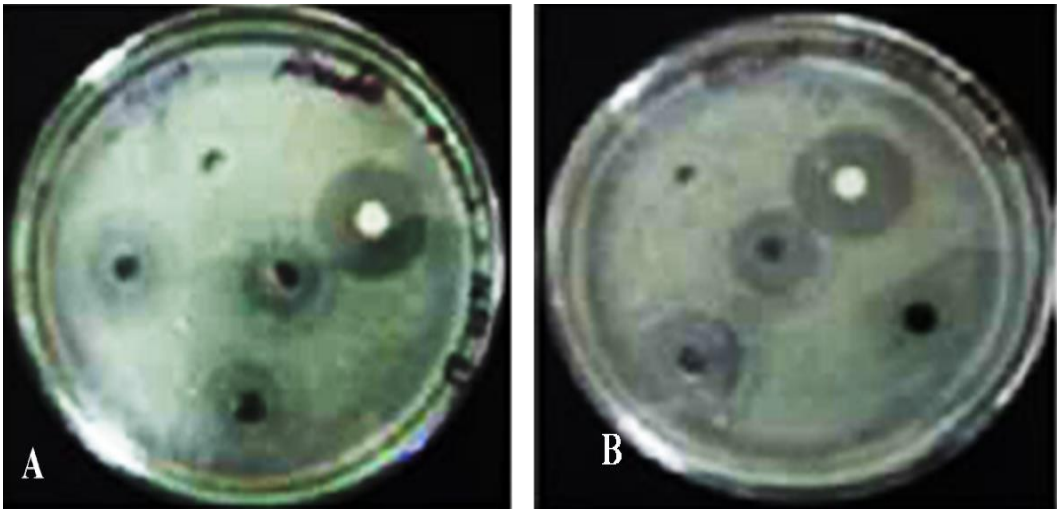

Figure 2. Antibacterial activity of *Streptomyces* spp. against pathogenic bacteria. **A.** Activity of secondary metabolites extracted against Gram-positive bacteria. **B.** Activity of secondary metabolites extracted against Gram-negative bacteria.

isolates showed varying degrees of inhibition against the tested pathogens. These findings suggested that the *Streptomyces* isolates collected from Hilla city possessed the potential to produce antimicrobial compounds. The antibiotics that are produced by *Streptomyces* serve as the sources of life saving environments and each type of these has specific biological function [19, 20]. *Streptomyces* are the major producers of important biomolecules, specially antibiotics that are used in treating a variety of diseases [21]. The results of this study agreed with Kumar *et al*. who collected *Streptomyces* *StrA* ins from the wasteland alkaline and garden soils in India and tested their extracts against pathogenic bacteria with the results showing antibacterial activity [22].

Identification of genes in *Streptomyces* isolates

The ability of *Streptomyces* isolates to produce the *StrA* and *StrB* genes involving in streptomycin biosynthesis was shown in Table 2. The J-C-93 isolate possesses both *StrA* and *StrB* genes, while the M-S-29 isolate possesses only the *StrB* gene. The remaining isolates (k-S-7, N-S-25, MA-S-90, M-S-48, H-S-66, and H-S-75) did not show

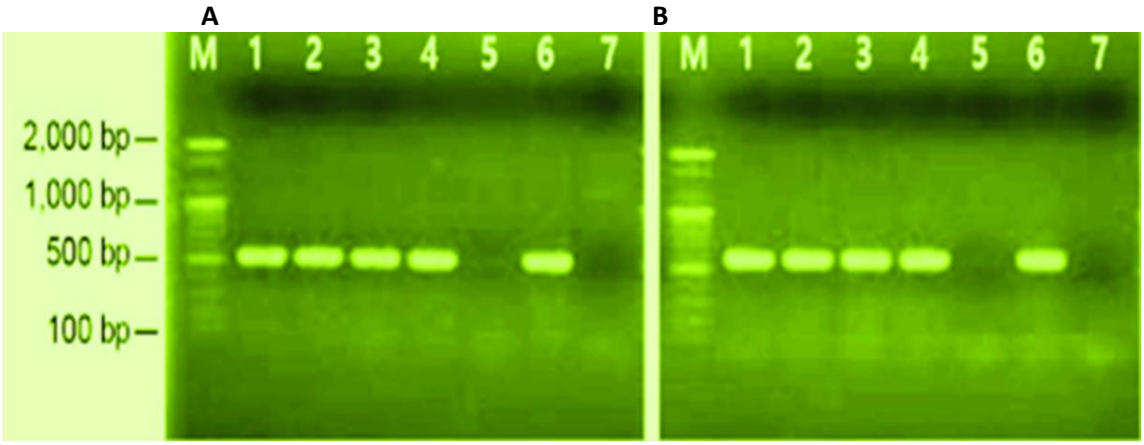

Figure 3. PCR amplification of J-C-93 isolate that produced *StrA* and *StrB* genes. **A.** Lanes 1-7: bands of *StrA* gene. **B.** Lanes 1-7: bands of *StrB* gene. Lanes M: 100-2,000 bp DNA ladder.

evidence of *StrA* or *StrB* gene expression. The ability of the J-C-93 isolate to produce streptomycin was confirmed by PCR reaction with the bands corresponding to the *StrA* and *StrB* genes being detected at 500 bp (Figure 3).

Table 2. *Streptomyces* spp. isolates that produced streptomycin by *StrA* and *StrB* genes.

***Streptomyces* spp isolates**	**Genes**	
	StrA	***StrB***
k-S-!7	-	-
N-S-25	-	-
MA-S-90	-	-
M-S-48	-	-
H-S-66	-	-
H-S-75	-	-
M-S-29	-	+
J-C-93	+	+

The results were consistent with previous research by Huddleston *et al*. who reported the ability of the J-C-93 isolate to produce streptomycin through the expression of both streptomycin phosphotransferase gene (*StrA*) and amidinotransferase (*StrB*), a key enzyme involved in streptomycin synthesis [23, 24]. Extensive research has characterized the biosynthesis of streptomycin, highlighting the crucial role of the *StrB* gene that is highly conserved in *Streptomyces* griseus and is essential for streptomycin production, regardless of whether the *StrA* gene is present. To isolate streptomycin, bacterial cultures are typically filtered using Whatman No. 1 filter paper, and the cell-free supernatant is obtained by centrifugation at 6,000 rpm for 15 minutes [23, 24]. Previous studies showed that *Streptomyces glaucescens* produced hydroxystreptomycin, while *Streptomyces bluensis* produced bleomycin [24].

Streptomyces bacteria isolated from local soils are often distinguished by their ability to produce streptomycin. Laskaris *et al*. isolated streptomycin from locally sourced soil samples [25]. In this study, the *StrA* and *StrB* genes responsible for streptomycin production were successfully amplified using PCR technology, confirming the ability of the isolates to produce this antibiotic. Furthermore, the produced streptomycin showed effectiveness against a range of pathogenic bacteria. These findings highlighted the potential of *Streptomyces* isolates collected from different regions in Hilla city, Babylon, Iraq to produce streptomycin. The J-C-93 and M-S-29 isolates, which possess both *StrA* and *StrB* genes involved in streptomycin biosynthesis, exhibited significant inhibitory activity against a range of Gram-positive and Gram-negative pathogenic bacteria. The results

suggested that these *Streptomyces* isolates held promise as a source of novel antimicrobial agents, warranting further investigation into the characteristics of the streptomycin they produce and their efficacy against a broader spectrum of pathogens.

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