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Full Length Article

Antimicrobial and Molecular Characterization of *Streptomyces* Species Isolated from Agricultural Soil of Jordan Valley

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Abstract

Antibiotic-resistance is growing around the world and is now a serious health concern. It is thus essential to find novel, extremely potent antibiotics. The *Streptomyces* genus has attracted the attention of researchers worldwide because it is the most well-known producer of antibiotics. This study aimed to isolate *Streptomyces* strains from the Jordan Valley soil and evaluate their ability to produce bioactive substances. Twenty bacterial strains were isolated, and the antimicrobial activity was performed against five types of pathogenic bacteria (*Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Escherichia coli* and *Pseudomonas aeruginosa*) using the modified cross-streak method for primary screening and the agar diffusion assay for secondary screening. One isolate, the SR2 strain, showed the highest bioactivity in the primary and secondary screenings against two pathogenic bacteria (*B. subtilis* and *P. aeruginosa*). It caused zones of inhibition of about 17–28 mm at 500 µg disc^{-1}. Therefore, this bacterial strain was selected for further studies. 16S rDNA sequencing was used to identify the SR2 strain. The identification findings revealed a 98% similarity to *Streptomyces labedae*. Overall, this study demonstrated that *Streptomyces labedae* SR2 isolated from Jordan Valley soil has the potential to produce effective antimicrobial compounds against both Gram-positive and Gram-negative pathogenic bacteria.

Key words: 16S rDNA; Antimicrobial activity; Jordan valley; Soil; *Streptomyces*

Introduction

Antibiotic-resistance is currently recognized as one of the most significant global health issues, as it has become a cause of a large number of deaths among patients infected with resistant bacteria that do not respond to antibiotics (Murray *et al*. 2022). Antibiotic-resistant strains spread easily and widely when antibiotics are misused and without the need for them in the light of natural evolution of bacteria (Muteeb *et al*. 2023). Bacteria evolve and become resistant to antibiotics naturally, such as by acquiring resistance genes from other bacteria by genetic transfer or through mutations (Urban-Chmiel *et al*. 2022). Therefore, it has become very necessary to search for new antibiotics with high activity to confront the problem of increasing antibiotic resistance (Nwobodo *et al*. 2022). Soil is one of the most significant sources of biologically active microorganisms in nature (Tripathi and Gaur 2021). The soil is rich in nutrients, which creates a state of intense competition for these resources among soil-dwelling organisms (Nekhili *et al*. 2021).

Streptomyces is a type of Gram-positive, mesophilic, filamentous, and aerobic bacterium that is a predominant presence in the soil, constituting up to 50% of the soil's bacterial population (Devi *et al*. 2022; Elias *et al*. 2022). It is considered the most productive bacterial genus for biologically active secondary metabolites (Selim *et al*. 2021). *Streptomyces* species has been well studied and described because of its significance in the biosynthesis of numerous significant pharmaceuticals, including antibacterials, antifungals, antivirals and antitumorals (Hamed *et al.* 2024). Remarkably, almost two-thirds of all known antibiotics are produced by *Streptomyces* species (Krause *et al.* 2019; Risdian *et al.* 2019). The present study was carried out to investigate the presence of biologically active *Streptomyces* species in the soil of the Jordan Valley.

Materials and Methods

Collection of soil samples

The Jordan Valley region, Jordan, was chosen to isolate soil *Streptomyces* strains. Three soil samples were collected from each of the following sites: the rhizosphere and around three meters from the roots. The samples were collected down to a depth of 15 cm after the top 3 cm of soil was removed.

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Isolation and cultural purification of *Streptomyces*

Soil samples were subjected to serial dilution up to a concentration of 1×10^{-6}. An aliquot of 100 μL from each dilution was plated onto ISP4 agar medium and incubated at 28°C for a period of five days. Pure cultures of bacterial isolates were obtained after selection based on phenotypic characteristics, as reported by Arifuzzaman *et al*. (2010). In detail, a single isolated colony from each growth plate was streaked on ISP4 agar media and incubated for five days at 28°C. Subsequently, the morphological properties of the bacterial isolates were examined using a stereomicroscope (Nikon, Japan).

Assessment of antimicrobial activity of *Streptomyces* isolates

Primary and secondary antimicrobial assays were performed for 20 *Streptomyces* isolates against five types of pathogenic bacteria (Table 1). The primary screening was carried out for the isolates by the modified cross-streak method (Selvin *et al*. 2009). Each *Streptomyces* isolate was grown in vertical lines on nutrient agar media plates and incubated for five days to allow it to produce the active products. The test pathogenic bacteria were streaked in horizontal lines near the vertical line and the plates were incubated for 18 h at the proper temperature. After that, the secondary screening of the *Streptomyces* isolates was performed using agar diffusion assay depending on the procedure of the Clinical and Laboratory Standards Institute and as detailed by Al-Qaralleh *et al*. (2021). To extract the bacterial crude metabolites, the bacterial isolates were inoculated separately in 500 mL of nutrient broth in 1 L Erlenmeyer flasks and incubated in an orbital shaker device (Jhohch, Korea) for seven days at a temperature of 28°C. Bacterial growth was monitored by recording optical density readings at 600 nm wavelength (UV-Vis spectrophotometer, LKB Biochrom, England). Once the bacteria reached the death stage, the bacterial broths obtained from the cultures of *Streptomyces* isolates were collected and subjected to centrifugation at 4,000 rpm for 30 min. The resulting supernatants were extracted by adding an equal volume of ethyl acetate. Following this, it was concentrated using a vacuum evaporator (Büchi Rotavapor R-215, Switzerland) at a temperature of 45ºC after drying over sodium sulphate. Following that, methanol was used to dissolve the crude extract at a concentration of 50 mg mL^{-1}. Then, it was kept at 4°C.

The minimum inhibitory concentration (MIC) of crude extract of the SR2 isolate was evaluated in triplicate using the micro-broth serial dilution assay following the Clinical and Laboratory Standards Institute method (CLSI 2008).

Biochemical tests and Gram staining of SR2 isolate

Gram stain carried out on the SR2 isolate, and the result was observed using a light microscope (Olympus Corporation, China). Indole synthesis, catalase activity, citrate consumption and melanin production assays were performed as outlined in Al-Saadi *et al.* (2013) for determining the biochemical characteristics of the SR2 isolate.

Table 1: bacterial strain used in antimicrobial activity assay

Name of bacteria	Strain No.
Gram positive bacteria	
Staphylococcus aureus	ATCC 43300
Bacillus subtilis	ATCC 6633
Bacillus cereus	ATCC 11778
Gram negative bacteria	
Escherichia coli	ATCC 25922
Pseudomonas aeruginosa	ATCC 27853

Molecular characterization of SR2 isolate

The genetic material of the SR2 isolate was extracted using GeneElute™ bacterial genomic DNA miniprep kit (Sigma-Aldrich, USA) according to the manufacturer's guidelines with a few adjustments. Polymerase chain reaction (PCR) was used to amplify 16S rDNA using universal forward and reverse primers. Gel electrophoresis was performed to ensure the PCR product was roughly 1,500 bp. The 16S rDNA was sequenced by Macrogen Inc. (South Korean). The result of sequencing was compared with data in GenBank to identify the isolated bacterial strain.

Results

Morphology features of *Streptomyces* isolates

A total of 20 *Streptomyces* strains were obtained from two distinct soil samples. The colonies were selected on the basis of their visible characteristics. They formed hyphae and conidia, or sporangia-like structures similar to fungi, and exhibited a powdery texture. They also stuck firmly to the ISP4 agar surface (Fig. 1).

Antimicrobial activity of *Streptomyces* isolates

As mentioned previously, every bacterial isolate was subjected to primary and secondary antimicrobial activity screening against pathogenic test microorganisms. The antimicrobial activity screening showed that the isolate SR2 was the most biologically active among all isolates. It inhibited the growth of two types of pathogenic bacteria (*Bacillus subtilis* and *Pseudomonas aeruginosa*) from growing permanently on agar media in primary screening (Fig. 2). As for the secondary test, the SR2 isolate exhibited significant bioactivity against the same pathogenic bacteria in the primary screening, as it caused an inhibition zone of (14–28) mm as shown in (Table 2). The Inhibition zone on the agar plate is illustrated in (Fig. 3).

Based on the agar diffusion assay result, MIC test was carried out and the growth inhibitory effects of the crude extract of the SR2 isolate against the test microorganisms are shown in (Table 3). Streptomycin was used as a positive control.

Table 2: Inhibition zones (mm) against two test bacterial strains caused by the SR2 isolate crude extract at different concentrations

Test strains	Crud extract concentrations (μg disc^{-1})		
	500	300	100
	Inhibition zone (mm)		
Bacillus subtilis	17 ± 0.1	15 ± 0.1	14 ± 0.1
Pseudomonas aeruginosa	28 ± 0.1	25 ± 0.1	20 ± 0.1

mean ± SD, n = 3

Table 3: Minimum inhibitory concentration (MIC) of SR2 strain crude extract tested on two pathogenic bacteria

Sample	*Bacillus subtilis*	*Pseudomonas aeruginosa*
Crude extract 50 μg mL^{-1}	125 S	125 S
Streptomycin 10 μg mL^{-1}	< 0.39 C	12.5 C

S: bacteriostatic; C: bactericidal

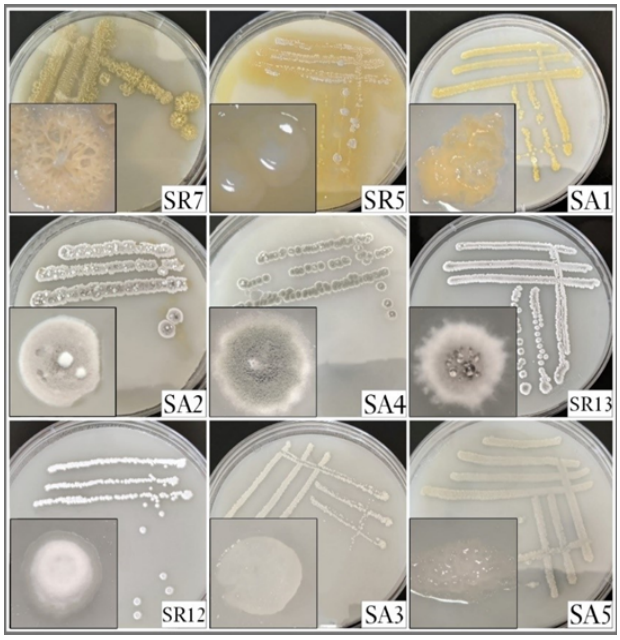

Fig. 1: Representative morphology and color of *Streptomyces* isolates on ISP4 media. Selected isolates are shown to highlight variation in appearance on ISP4 medium

The SR2 isolate reached its maximum growth after seven days and showed dense growth, white spores, and a rough surface on ISP4 agar examined using an Olympus CX2 stereomicroscope. After performing biochemical assays, the results revealed that the SR2 isolate was negative for the indole test, citrate utilization, melanin production (production of deep brown to black diffusible pigment) and catalase test.

To identify the SR2 isolate, the 16S rDNA gene sequence analysis was carried out. The analysis result showed 98% similarity to the *Streptomyces labedae*. The phylogenetic tree was constructed by aligning the 16S rRNA gene sequence of *Streptomyces labedae* strain SR2 with those of homologous sequences from other *Streptomyces* species (Fig. 4).

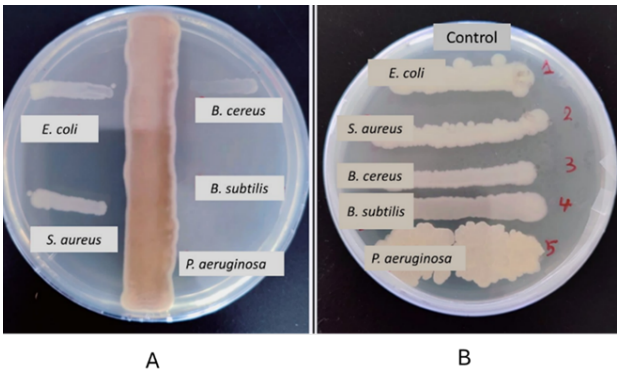

Fig. 2: Antimicrobial activity of SR2 isolate using the modified cross streak method on a nutrient plate. Panel **A** shows the bioactivity of SR2 isolate against test microorganisms; Panel **B** serves as a negative control

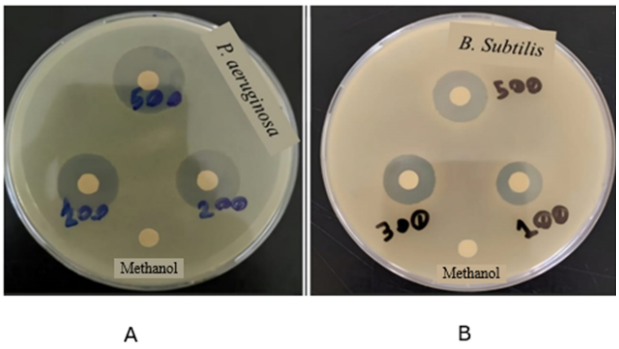

Fig. 3: Agar diffusion assay of SR2 isolate crude extract. Antimicrobial activity of the SR2 strain crude extract against (**A**) *P. aeruginosa* and (**B**) *B. subtilis*

Discussion

Antimicrobial resistance can affect people of all ages, as well as the healthcare, veterinary, and agricultural industries (Dadgostar, 2019). Consequently, it has become one of the world's most serious public health concerns (Salam *et al*. 2023). Therefore, the only possible solution is to expand the effort of researchers in searching for novel, potent antibiotics (Schneider 2021).

In this study, 20 isolates of the soil *Streptomyces* were isolated from the Jordan Valley region. The soil samples were obtained from the rhizosphere and at a distance of about 3 meters from the plant root. The Jordan Valley was selected as a collection site because it is considered an extreme environment, specifically a hot and dry climate (Bitan 1982). Such harsh environments are known to enhance the production of antimicrobial compounds by Actinomyces (Fatahi-Bafghi *et al*. 2019). Thereby, providing a potential source for novel *Streptomyces* isolates.

The 20 bacterial isolates were subjected to the primary and secondary antimicrobial screenings, and the results revealed that there was no correlation between the bioactivity of some bacterial isolates on a nutrient plate and

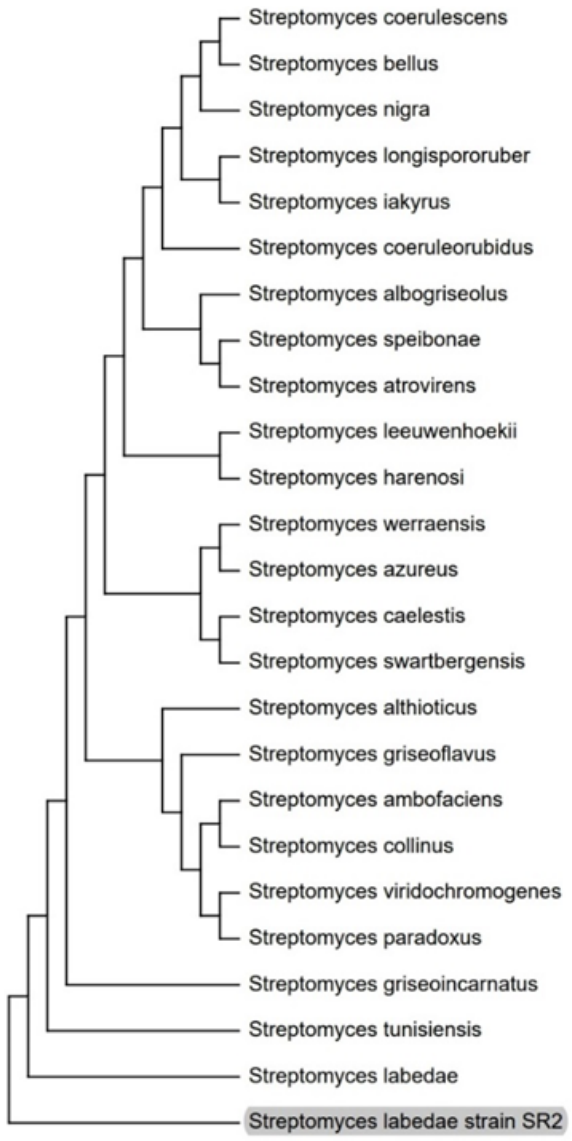

Fig. 4: Phylogenetic tree illustrating the relationship between *S. labedae* strain SR2 (highlighted in gray) and 25 *Streptomyces* spp.

their extracts in the nutrient broth. In the preliminary screening, for instance, *S. labedae strain SR2* showed antimicrobial activity against *B. cereus*, *B. subtilis* and *P. aeruginosa*. However, its extract did not exhibit any antibacterial activity against S. cereus in the secondary screening.

Antimicrobial activity against *P. aeruginosa* and *B. subtilis* was found by *S. labedae* SR2. Due to its single layer of peptidoglycan and absence of an outer lipid membrane, Gram-positive *B. subtilis* is more susceptible to secondary metabolites produced by other bacteria (Rajagopal and Walker 2017). However, the gram-negative *P. aeruginosa* showed relative resilience due to its existence that lipopolysaccharides are one of its main components (Kim and Hearnsberger 1994). Despite this, the SR2 isolate revealed the broad-spectrum potential of its bioactive chemicals by continuing to show considerable inhibitory action against *P. aeruginosa*.

The results of the biochemical assays showed that the SR2 isolate doesn't produce pigmented secondary metabolites on ISP4, is unable to use citrate, and cannot synthesize catalases and indole. A restricted metabolic pattern characteristic of certain bioactive *Streptomyces* species is reflected in the biochemical profile of SR2 (Chevrette *et al.* 2019; Xu *et al.* 2022). Notwithstanding these limitations, SR2 has considerable antibacterial activity, suggesting that neither pigment synthesis nor indole metabolism are necessary for the production of bioactive secondary metabolites (Li *et al.* 2024).

Conclusion

The findings of this study revealed that *Streptomyces labedae* SR2, isolated from agricultural soil, could produce substances with antimicrobial activity, especially against the Gram-negative bacterium *Pseudomonas aeruginosa*, which is known for its high resistance to antibiotics.

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Author Contribution

AHT planned the experiments. AHT and LRQ interpreted the results while AHT and LRQ made the write up.

Conflict of Interest

No conflicts of interest were declared by the authors.

Data Availability

The data can be obtained from the corresponding author upon a reasonable request.

Ethics Approval

Not applicable to this study.

Funding Source

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