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Original Research Article

Isolation, Antibacterial Screening, Phenotypic and Molecular Identification of Antimicrobial-producing Soil *Streptomyces* Strain

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ABSTRACT

The increasing prevalence of antibiotic-resistant bacteria has prompted a global search for new antibiotics. *Streptomycetaceae* is one of the major families that produce antibiotics for medicinal use. Researchers have investigated the genus *Streptomyces* to identify new species that could produce potent antibacterial substances. This investigation was conducted to isolate and characterize *Streptomyces* strains with antibacterial potential from different soil samples in the Al-Karak Governorate, Jordan. Soil samples were obtained from caves, home gardens, greenhouses, and agricultural farmlands in the Governorate. *Streptomyces* were isolated and identified from the various soil samples. The antibacterial activity of the *Streptomyces* isolates was examined against a group of test bacteria using primary (modified cross-streak method) and secondary (agar diffusion test) screening methods. The isolate with the highest antimicrobial activity was identified based on morphological, biochemical, and molecular characterization. The results indicated that twenty-four *Streptomyces* strains were isolated from the different soil samples, all showing antimicrobial potential against at least two of the test bacteria. One of the isolates, the C8 strain had the highest antimicrobial potential in the primary screening against all test bacteria and displayed activity in the secondary screening against four types of test bacterial strains. The sequence analysis of the 16S rDNA gene showed that isolate C8 was 98.7% similar to *Streptomyces pratensiss*. The present study's findings suggested that *Streptomyces pratensiss* strain C8 from cave soil could produce antibacterial compounds, perhaps supporting the search for naturally occurring bioactive medications.

***Keywords*:** Antimicrobial activity, Soil samples, *Streptomyces*, 16S rDNA, Jordan.

Introduction

Antimicrobial resistance poses a very high risk to global public health because common infections could become life-threatening, and common surgical procedures and other medical treatments could become more challenging if effective antimicrobials disappeared.[1] As a result, scientists are actively searching for novel, sustainable, effective, and wide-spectrum antimicrobial compounds from a variety of sources, including microorganisms found in natural soil environments.[2] Actinobacteria is one of the largest bacterial phyla, accounting for 13–30% of the soil microbiota.[3] It produces bioactive compounds, particularly antibiotics, which are crucial for treating diseases of public health concern.[4]

Streptomyces belongs to the actinomycetes and within the *Streptomycetaceae* family, it is the largest genus. The *Streptomyces* species produce the majority of bioactive secondary metabolites.[5]These bacteria are aerobic, Gram-positive, mesophilic, and filamentous, widespread in terrestrial and marine environments. However, their habitat diversity is greatest on land.[6]

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The abundance of secondary metabolites produced by *Streptomyces* to kill or inhibit their competitors, thus allowing increased access to space or resources in the soil, is likely due to the complexity of soil habitats and interactions between *Streptomyces* and other species.[7] This has led to periodic large-scale screening and isolation attempts due to its notable secondary metabolites in both medical and commercial aspects.[8]

The present study aimed to isolate, screen, and conduct phenotypic and molecular characterization of antimicrobial-producing soil *Streptomyces* strains.

Materials and Methods

Collection of soil samples

Several sources of soil samples in the Al-Karak Governorate in Jordan were selected for the isolation of *Streptomyces* species. Samples of soil were taken from the following sources: caves, home gardens, greenhouses, and agricultural farmlands. The samples were collected between September and October 2021.

Isolation of pure colonies of Streptomyces strains

To isolate the desired strains, 1 g of each soil sample was placed in 9 mL of sterile distilled H_2O and then serially diluted up to 1×10^{-6}. Then, 100 µL of an aliquot from each dilution was spread over ISP4 agar plates. The culture was incubated at 28°C for six days. Pure colonies of *Streptomyces* were obtained by streaking ISP4 agar media with a single isolated colony from every growth plate and incubating them for six days at 28°C. A stereomicroscope (Nikon, Japan) was used to examine the morphological features of a culture grown for 6 days on tryptone soya agar (TSA) plates.

Screening of the antibacterial activity of Streptomyces strains

The modified cross-streak method (MCSM) was employed for the preliminary screening to determine the antimicrobial activity of twenty-four *Streptomyces* isolates against test pathogenic bacteria (Table 1).[9] After that, the agar diffusion assay was used for secondary screening to determine the bacterial secondary metabolites following guidelines from the Clinical and Laboratory Standards Institute (CLSI).[10] To perform the agar diffusion assay, the bacterial isolates were cultured individually in 1L Erlenmeyer flasks with 500 mL of nutrient broth for 7 days in an orbital shaker device (Jhohch, Korea) at 150 rpm and 28°C. Daily measurements of pH using a pH meter (WTW, Germany) and optical density (OD 600 nm) using a UV spectrophotometer (LKB Biochrom, England) were carried out to track the growth of bacteria. The *Streptomyces* liquid culture was collected and centrifuged for 30 minutes at 4,000 rpm, following the onset of a fall in culture absorbance, which indicated the entry of the bacteria into the death phase. An equal volume of sodium sulphate and ethyl acetate was used to extract the supernatant, which was then concentrated at 45°C in a vacuum evaporator (BüchiRotavapor R-215, Switzerland). The resultant crude extract was dissolved in methanol at a concentration of 50 mg/mL and stored at 4°C. A micro-broth serial dilution assay was used to determine the minimum inhibitory concentration (MIC) of the bioactive C8 isolate crude extract following the Clinical and Laboratory Standards Institute (CLSI) procedures.

Gram staining and biochemical testing

Standard Gram staining was performed on the C8 isolate, and the results were observed using a light microscope (Olympus Corporation, China). Biochemical assays including melanin formation, citrate utilization, catalase, and indole synthesis tests were used to determine the biochemical features of the C8 isolate.

Molecular characterization of the C8 isolate

Isolation of genomic DNA was achieved using the GeneElute™ bacterial genomic DNA miniprep kit (Sigma-Aldrich, USA) following the manufacturer's instructions with some modifications. The 16S rDNA was amplified by polymerase chain reaction (PCR) using universal forward and reverse primers. Gel electrophoresis was used to validate the approximately 1,500 bp PCR result. Sequencing of the 16S rRNA gene was conducted by Macrogen Inc. (South Korean). The nucleotide sequence was analyzed by cross-referencing it with previously published bacterial DNA sequences in GeneBank.

Table 1: Test microorganisms used in the antimicrobial activity assay

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

Results and Discussion

Cultural characteristics of Streptomyces strains

A total of twenty-four *Streptomyces* isolates were obtained from four different soil samples. Colonies of *Streptomyces* species were selected based on their colony morphology because their colonies have a powdery texture, securely bonded to the ISP4 agar's surface, and produced hyphae,[11] as illustrated in Figure 1. Each isolate was streaked out with one colony on a solid medium of TSA for additional phenotypic characterization of the *Streptomyces* isolates. Following six days of incubation, the isolates showed a broad range of pigments, including white, yellow, light pink, and orange, which influence the colour of the vegetative and aerial mycelia (Figure 2).[12]

Antimicrobial potential of Streptomyces strains

As previously mentioned, all the *Streptomyces* isolates were subjected to a preliminary and secondary bioactivity screening against pathogenic bacteria. Isolate C8 was the only isolate that showed activity against all test pathogenic bacteria in the preliminary screening procedure (Figure 3 and Panel A), and a strong antibacterial inhibitory potential against four test microorganisms in the secondary screening method (Table 2 and Figure 3 Panel B). Therefore, it was selected for further study. The results of the bioactive screening demonstrated that, despite the widespread distribution of *Streptomyces* bacteria, environmental factors like temperature and nutritional viability regularly affect the population dynamics of these organisms.[13] Since cave soil is recognized for its extreme conditions (high humidity, reduced oxygen level, low temperature, and limited nutrient availability),[14] the C8 isolate had the most antibacterial activity compared to other isolated strains. Table 3 displays the results of the C8 isolate crude extract's growth inhibitory effects against the test bacteria based on the agar diffusion assay. Streptomycin was used as a positive control.

The identity of Streptomyces C8 isolate

The *Streptomyces* C8 isolate reached its maximum growth after six days of incubation, exhibiting off-white sporulation. Moreover, the surfaces of the spores were rough, glabrous, or chalky. Examined with an Olympus CX2 stereomicroscope, moderate to heavy growth on the ISP4 medium was observed (Figure 4). The biochemical tests revealed that the isolate of *Streptomyces* strain C8 was positive for citrate consumption and negative for the formation of melanin, indole, and catalase utilization. The strain was identified as a long filamentous Gram-positive bacterium. The Simmon citrate agar's colour changed from green to blue in the citrate utilization test, indicating that *Streptomyces* strain C8 could utilize citrate. However, the indole test, which showed a red ring on the surface of the broth, showed that the C8 isolate was unable to convert tryptophan to indole. Furthermore, this strain did not produce coloured secondary metabolites on the ISP4 medium. The 16S rDNA gene sequence analysis was used to identify the *Streptomyces* C8 isolate. It had 98.7% similarity with *Streptomyces pratensiss*. To generate a phylogenetic tree, *Streptomyces pratensiss* strain C8's 16S rRNA gene sequence was linked using multiple sequence alignments with homologs of other *Streptomyces* species (Figure 5).

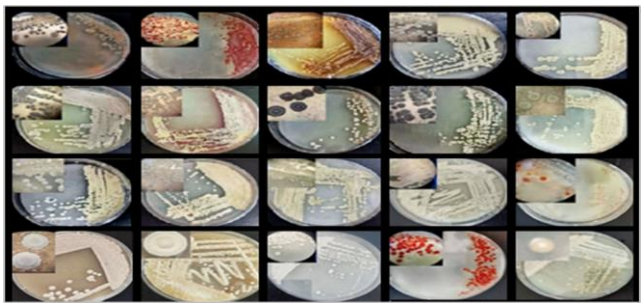

Figure 1: Colour and morphology of selected *Streptomyces* isolates on ISP4 plate

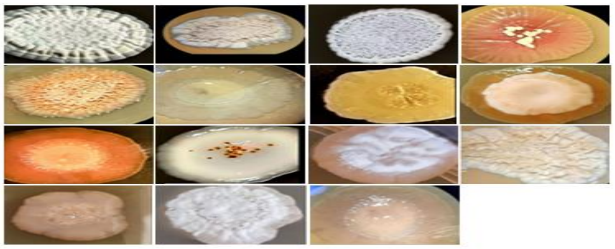

Figure 2: Colony morphology of selected *Streptomyces* isolates on TSA plate

Table 2: Inhibition zones caused by different concentrations of *Streptomyces* species C8 isolate crude extract

Test S-strain	Zone of inhibition (mm) at different concentrations		
	500 µg/disc	300 µg/disc	100 µg/disc
E. coli	11	ND	ND
B. cereus	13	8	ND
P. aeruginosa	16	10	ND
B. subtilis	13	7	ND

ND: Non-detection of bioactivity at this concentration.

Table 3: Minimum inhibitory concentration (MIC) of the crude extract from *Streptomyces* species C8 isolate against test bacteria

Sample	*P. aeruginosa*	*B. subtilis*	*B. cereus*	*E. coli*
Crude extract (50 µg/mL)	250s	500s	500s	500s
Streptomycin (10 µg/mL)	12.5c	0.39c	25c	6.25c

S: Bacteriostatic; C: Bactericidal

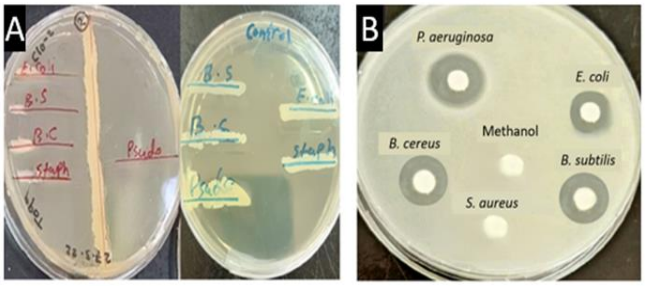

Figure 3: Antimicrobial activity of *Streptomyces* species C8 isolate

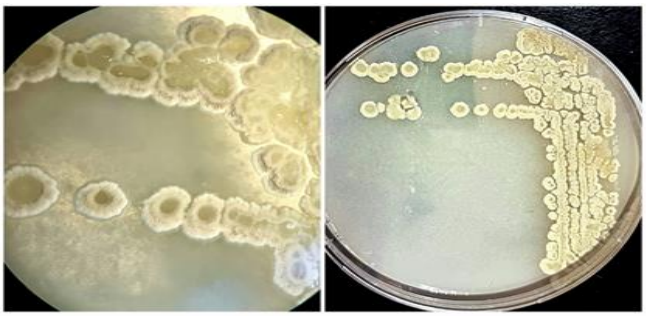

Figure 4: The phenotype of *Streptomyces* species C8 isolate on ISP4 plate

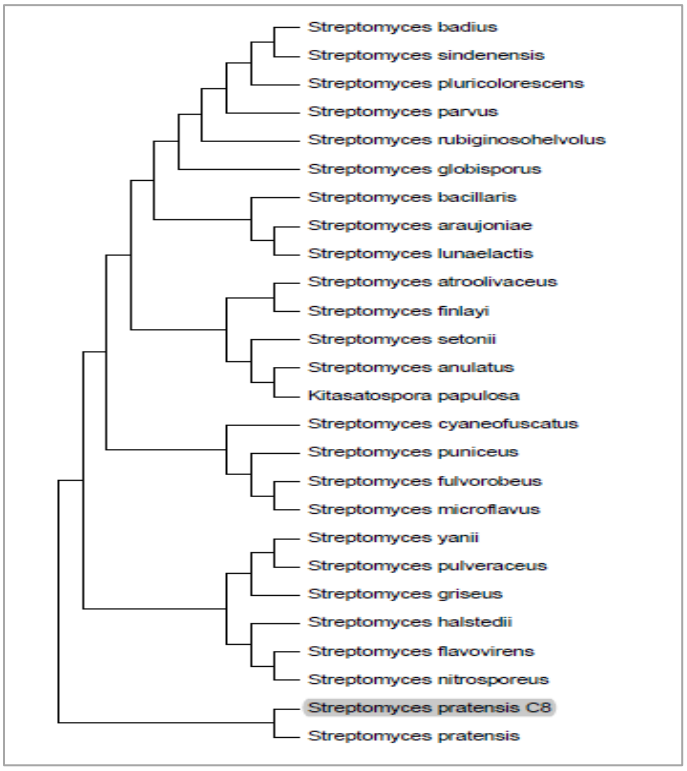

Figure 5: Phylogenetic tree representing relationships among twenty-five *Streptomyces* species and *Streptomyces pratensiss* strain C8

Conclusion

The findings from the present study revealed that the *Streptomyces pratensiss* strain C8 isolated from cave soil could produce antibacterial compounds that could be useful in many applications and should be extensively explored. Cave soil might be an imperative and excellent resource for finding naturally occurring bioactive medications.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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