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A New Source of Actinomycetes with Heavy Metal Tolerance and Antibacterial Properties Against Multidrug-Resistant *Staphylococcus hominis*

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Abstract

Objective: This study aimed to isolate copper (Cu)- and nickel (Ni)- tolerant actinomycetes with antibacterial properties against multidrug-resistant *Staphylococcus hominis* and *Escherichia coli*.

Methods: Bacteria were isolated from soil samples of the floor and ceiling of Umm Jirsan Cave using starch nitrate agar medium. The isolates were identified using 16S rRNA, and their tolerance to heavy metals at various concentrations (50, 100, 150, 200, 250, and 300 mg/L) was screened, and the antibacterial activities were detected using an agar plug method. Also, the antibacterial potential of metal-resistant bacteria was further examined on metal-embedded media at the lowest concentration tested (50 mg/L).

Results: Ten different bacterial strains were isolated, identified, and characterized for their tolerance to Cu and Ni and their antibacterial activity against the bacterial pathogens, *S. hominis* and *E. coli*. Phylogenetic analysis of the strains confirmed that nine of them belong to Gram-positive bacteria, Actinobacteria phylum, *Streptomyces* genus, based on their shared 99–100% identity with known *Streptomyces* species. Only one isolate was a Gram-negative bacterium identified below the Proteobacteria phylum as *Sphingomonas ursincola* C6 (100% similarity). The results revealed a varying response of the bacterial species to the two heavy metals. At the highest tested concentration of metals (300 mg/L), 40% of the isolates showed Ni-resistance, and 10% of isolates were Cu-resistant, suggesting that these cave bacteria were more resistant to Ni than Cu. In addition, six isolates demonstrated good antibacterial potential against *S. hominis*, with inhibition zone diameters ranging from 11.0 ± 0.59 to 21.5 ± 0.5 mm, with the highest values, 21.5 ± 0.5 and 21.0 ± 0.63 mm, for *Streptomyces vinaceusdrappus* C2 and *Streptomyces huasconensis* C8, respectively. Interestingly, the presence of Cu (50 mg/L) in the growth medium significantly enhanced the antibacterial activity of *S. vinaceusdrappus* C2 against *S. hominis*, with an inhibition zone of 27.5 ± 0.5 mm. Similarly, *S. huasconensis* C8 recorded a higher inhibition zone of 27 ± 0.23 mm against *S. hominis* in the medium supplemented with Ni (50 mg/L). No inhibition zones against *E. coli* were recorded by the tested bacteria.

Conclusion: The results conclude that bacteria, especially actinomycetes, from Umm Jirsan Cave are valuable candidates for pharmaceutical research and bioremediation, owing to their metal-tolerance and antibacterial properties.

Keywords: Cave, bacteria, drug resistance, bacterial, microbial sensitivity tests, Saudi Arabia

Introduction

Some heavy metals are essential micronutrients required for biochemical and physiological functions in living organisms, but in large concentrations they can become toxic.[1] Heavy metals pose a serious threat to both human health and the ecosystem due to their nondegradable nature, potential toxicity, and bioaccumulation.[2] Given the severe effects of heavy metals, urgent actions are required to eliminate them from polluted environments. While physicochemical techniques like membrane filtration, precipitation, oxidation, and reduction exist, they have limitations.[3] Microorganisms play a crucial role in bioremediation because of their rapid growth and ease of manipulation.[4] Metal microbial remediation stands out as a highly efficient, cost-effective, and environmentally friendly approach to detoxifying metals.[5]

On the other hand, according to the World Health Organization (WHO), antimicrobial resistance (AMR) poses an important global health challenge and is one of the top threats facing humanity.[6] As soon as a new antibiotic receives approval for widespread clinical use, its efficacy declines over time due to bacterial evolution of resistance.[7] The overuse and misuse of antibiotics have hastened the spread of antibiotic-resistant bacteria, making it harder and more costly to treat infections.[8] If solutions are not urgently implemented, by 2050, AMR will perhaps be responsible for 10 million deaths annually.[9] This crisis would also incur economic losses reaching over $100 trillion.[10] Therefore, medication development is a key strategy for preventing infectious disease epidemics and reducing morbidity and mortality, and searching for natural and effective drug sources is a pressing need.

Cave ecosystems represent underexplored reservoirs for microbial exploration, which host diverse, especially extremophilic microorganisms. Bacteria that adapt to nutrient-poor, aphotic environments produce remarkable novel secondary products, such as antibiotics and enzymes, which hold significant promise for therapeutic and industrial use.[11] Among cave microbiomes, actinomycetes are recognized for their ability to produce diverse and valuable natural products for biotechnological applications.[12] Additionally, specific bacterial species have developed impressive strategies such as biosorption, bioaccumulation, and enzymatic transformation of metals into less harmful forms to manage heavy metals.[13] In recent decades, cave actinomycetes have attracted increased attention due to their potential to produce novel bioactive compounds that exhibit antibacterial, antioxidant, and anticancer properties (Farda et al., 2022).[14]

In the present study, research on the untapped harsh-environment Umm Jirsan Cave (northwestern Saudi Arabia) would contribute to discovering unique bacterial species with heavy-metal tolerance and production of valuable secondary metabolites, providing candidates for environmental bioremediation and sustainable antimicrobial agents against multidrug-resistant pathogens.

Materials and Methods

Study Area

The environmental samples were collected from the Umm Jirsan Cave lava tube, situated at coordinates 25.5888°N, 39.7570°E, and an elevation of 1185 m above sea level. The cave lies within the Harrat Khaybar volcanic field (Figure 1), approximately 125 km north of Madinah in western Saudi Arabia. Notably, it holds the title for the longest lava tube in Arabia, extending a remarkable 1481 m in a horizontal passage. The typical height of the passages ranges from 8 to 12 m, while the maximum width reaches up to 45 m. The lava tube system comprises three distinct passages, which are divided by two collapses, with the main tunnels branching east and west of Collapse 1.[15]

Isolation of Actinomycetes

Three soil samples were obtained from different locations within Umm Jirsan Cave, including the floor and the ceiling. All samples were preserved in the laboratory at 4°C until used. One gram of each soil sample was taken and serially diluted to 10^{-4} by distilled water, and the mixtures were shaken vigorously using a vortex. Thereafter, 0.5 mL of the final dilution was placed on starch nitrate agar medium,[17] and the inoculum was spread evenly using a sterile glass spreader. The inoculated plates were incubated at 28°C for 7 days.[18] Then, bacterial strains that showed morphological differences were selected and isolated by subculture in the same medium to obtain pure colonies. The purified isolates were stored at 4°C on starch nitrate agar slants; for long-term storage, suspensions of pure cultures mixed with 50% (v/v) glycerol were stored at –20°C.[19]

Identification of Bacterial Isolates

DNA Extraction and 16S rRNA Gene PCR Amplification

Genomic DNA extraction was performed at the laboratories of the Department of Biological Science, Faculty of Science, King Abdulaziz University, Saudi Arabia. The isolates were cultured in Luria-Bertani (LB) broth for 24 hr. Following incubation, 10 mL of each bacterial culture was centrifuged at 7,500 rpm for 10 min to pellet the cells. Then, the cell pellets were resuspended in 300 µL of lysis buffer (TES) consisting of 100 mM Tris base (pH 8), 50 mM EDTA, and 10% SDS. The resuspended cells were then transferred to 1.5 mL microcentrifuge tubes, 20 µL of lysozyme (20 mg/mL) was added, and the mixture was incubated at 37°C for 2 hr. After that, 20 µL of proteinase K (20 mg/mL) was added, and the tubes were incubated at 37°C for an additional 2 hr. To the lysed cells, 300 µL of sodium acetate (4 M) was added, gently mixed, and then incubated at –20°C for 10 min. Following this step, 500 µL of chloroform: isoamyl alcohol mixture (24:1 ratio) was added, and the solutions were mixed gently for 10 min by inversion. The mixture was then centrifuged at 12,000 rpm for 15 min to separate the phases. The upper aqueous phase containing the DNA was carefully transferred to a sterile 1.5 mL microcentrifuge tube, ensuring no contamination from the interface layer. To precipitate the DNA, an equal volume of isopropanol (precooling at –20°C) was added to each tube and mixed gently by inversion. The tubes were then incubated at –80°C for 1 hr to facilitate DNA precipitation. After incubation, the tubes were centrifuged at 12,000 rpm for 15 min to pellet the DNA. The supernatants were discarded, and the DNA pellets were washed with 70% ethanol to remove residual salts. Care was taken to decant the ethanol thoroughly, and the DNA pellets were air-dried at room temperature or in a 50°C incubator. It is crucial not to over-dry the pellets, as this may hinder their dissolution in the subsequent step. Finally, the DNA pellets were resuspended in 50 µL of Tris-EDTA (TE) buffer and were allowed to sit overnight at 4°C to ensure complete dissolution. The purity of DNA was assessed through the A260/A280 ratio using the NanoDrop 7000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Additionally, DNA integrity was verified using 1% agarose gel electrophoresis and ethidium bromide (EtBr) staining.

Fig. 1 **The map shows the location of Harrat Khaybar (red rectangle), the region's largest and most complex geological field.**[16]

The amplification of 16S rRNA was carried out using a thermal cycler (Applied Biosystems Veriti™ 96-Well Thermal Cycler provided by Macrogen in Seoul, Korea). The process utilized universal forward and reverse primers, 27F (5' AGAGTTTGATCMTGGCTCAG 3') and 1100R (3' GGGTTGCGCTCGTTG 5'). The reaction mixture was prepared at a volume of 50 µL, comprising 1 µL of DNA template, 1 µL of primers, 25 µL of green PCR mix (Promega, Go Taq R Green Master Mix, USA), and 22 µL of RNase-free water.

The PCR technique involved an initial denaturation step at 95°C for 5 min, followed by amplification, which includes three steps (repeated 36 times). The three steps were denaturation at 94°C for 40 sec, annealing at 58°C for 40 sec, and extension at 72°C for 90 sec. The PCR process concluded with a final extension at 72°C for 10 min. To evaluate the amplification product, 1% agarose gel electrophoresis was performed, and gels were visualized using the Ultra-Violet Product (UVP BioSpectrum® Imaging System).[20,21]

Sequencing and Phylogenetic Analyses

The amplified PCR products of the bacterial gene fragments were sequenced at Macrogen, Inc. (Seoul, Korea). The obtained sequences were compared with the National Center of Biotechnology Information (NCBI) database through the Basic Local Alignment Search Tool (BLAST) program. To identify the isolates, sequences of type strains most closely related to the isolates' sequences were searched.[22]

Screening of Isolates for Heavy Metal Resistance

The isolated bacteria were investigated for their tolerance to two heavy metals: copper (Cu) and nickel (Ni). Stock solutions of the tested heavy metals were prepared by adding 5 g of the selected material to 1000 mL of distilled water. The copper stock solution was made from copper sulfate ($CuSO_4$), and the stock of nickel was prepared from nickel (II) chloride hexahydrate ($NiCl_2{\cdot}6H_2O$). The heavy metal solutions were sterilized using a sterile syringe filter with polyethersulfone (PES) membrane (pore size: 0.45 μm) and then preserved at 4°C until used. Screening for heavy metal-resistant bacteria was performed using six metal concentrations (50, 100, 150, 200, 250, and 300 mg/L) added separately into the sterilized media.[23] The bacterial isolates were cultivated on the prepared media, and the inoculated plates were incubated at 28°C for 14 days. After the incubation period, bacterial growth was followed to assess their ability to survive in various metal concentrations, and the highest concentration of each metal supporting bacterial growth was defined as the maximum tolerance level (MTL).

Screening of Isolates for their Antibacterial Activity

The production of antimicrobial compounds by the chosen bacteria was assessed through the agar plug method.[24] The following two indicator cultures were used: *Staphylococcus hominis* and *Escherichia coli* (from the Biological Department Culture Collection, which are resistant to two different antibiotics).

The tested isolates were spread separately on starch nitrate agar plates. All plates were incubated at 28°C for 8 days for better growth and antibiotic production. Suspensions of overnight-grown indicator cultures were smeared onto the surface of Mueller-Hinton agar plates. A sterile cork borer measuring 5 mm in diameter was used to remove agar discs of bacteria, which were then placed onto inoculated Mueller-Hinton agar media.[25] The plates were left at room temperature for 2 hr to allow the diffusion of antibiotic compounds from the discs. Subsequently, the plates were incubated at 37°C for 24 hr.[26] After incubation, the diameter of the inhibition zone, including the disc itself, was measured with a ruler and recorded in mm to evaluate the antibacterial potential of the tested isolates, as a mean ± SD of triplicate measurements. If no inhibition zone was observed, it was recorded as a negative result.

Additionally, the actinomycete isolates that were both metal-resistant and inhibited the growth of *S. hominis* were selected to assess their antibacterial activity when grown on modified starch nitrate agar media containing 50 mg/L of either Cu or Ni. Discs of modified media without an inoculum were used as controls to ensure that the pathogen growth inhibition was caused only by the tested actinomycetes, not the metal itself. The susceptibility of *S. hominis* to the actinomycete isolates was evaluated by calculating the average readings of inhibitory zones.

Statistical Analysis

Data obtained from this study were analyzed as needed, depending on the nature of the data received. The screening experiments of metal-resistant bacteria were conducted three times to ensure the reliability and accuracy of the results. Statistical analysis of antibacterial activity, which involved measuring inhibition zone diameters, comprised calculating the mean and standard deviation (SD) of three replicates. Differences in mean values were considered significant at $P < 0.05$.

Results

Cave Isolates

A total of 10 bacterial isolates were obtained from different samples collected from Umm Jirsan Cave and coded as C1–C10. Table 1 shows the isolation areas and designations of the strains. Pictures of pure cultures of some isolates on starch nitrate agar medium, showing colonies' morphology and pigment production, are displayed in Figure 2.

Molecular Identification of the Isolates

The obtained sequence data were aligned and subjected to a phylogenetic analysis and homology examination. The 16S rRNA analysis revealed that most of the isolates (90%) were classified within the phylum Actinobacteria, containing high GC DNA content (57–59%). It was noticed that all strains were most closely related to the genus *Streptomyces*, belonging to different species. However, one isolate, named C6, was identified as *Sphingomonas ursincola* C6 (not an actinomycete), which is a Gram-negative, rod-shaped bacterium with a GC content of 51%. Table 2 illustrates bacterial identification by 16S rRNA gene sequencing, with GenBank accession numbers

Table 1. **Number of strains isolated from diverse soil samples of Umm Jirsan Cave**

Sample no.	Location of sample	Number of isolates
1	The entrance floor at a depth of 10 cm	4 (C1 to C4)
2	The floor inside the cave at a depth of 5 cm	4 (C5 to C8)
3	The ceiling	2 (C9–C10)

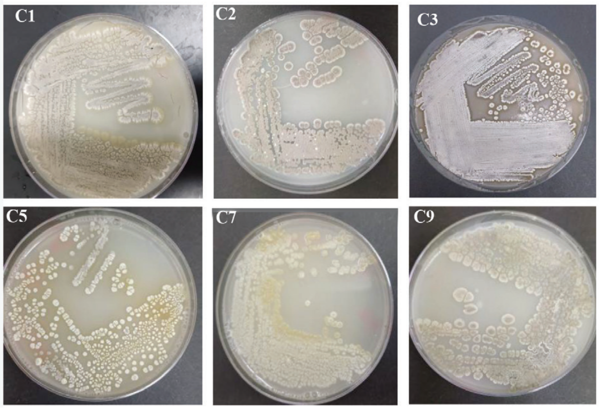

Fig. 2 **Morphological appearance of some bacterial colonies isolated from Umm Jirsan Cave, after 7 days of incubation on starch nitrate agar at 28°C.**

Table 2. **Molecular identification of isolated bacteria based on 16S rRNA gene sequence analysis**

The isolate	Accession number	The closest species	Similarity (%)	GC content
C1	PV798845	*Streptomyces labedae* strain CSSP735	100	58.0
C2	PV798846	*Streptomyces vinaceusdrappus* strain NBRC 13099	100	58.0
C3	PV798847	*Streptomyces thermocarboxydus* strain NBRC 16323	100	59.0
C4	PV798848	*Streptomyces harenosi* strain PRKS01-65	99.01	58.0
C5	PV798849	*Streptomyces griseorubens* strain NBRC 12780	100	57.0
C6	PV798850	*Sphingomonas ursincola* strain DSM 9006	100	51.0
C7	PV798851	*Streptomyces griseoflavus* strain LMG 19344	99.40	58.0
C8	PV798852	*Streptomyces huasconensis* strain HST28	99.70	57.0
C9	PV798853	*Streptomyces griseoincarnatus* strain NBRC 12871	100	58.0
C10	PV798854	*Streptomyces coelicoflavus* strain CSSP410	99.60	58.0

for the isolates. The existing similarity pattern among cave bacterial strains and their nearest relatives is presented in the phylogenetic tree provided in Figure 3 (A and B).

Copper (Cu) and Nickel (Ni) Resistance of Isolates

To determine heavy metal resistance, bacterial growth on starch nitrate agar supplemented with various concentrations of metals was recorded after 14 days of incubation at 28°C. The presence of significant growth indicated tolerance of the heavy metals tested. In Figure 4, the results show that a single strain, *Sphingomonas ursincola* C6, was capable of growing with all the tested concentrations of Cu and Ni. The greater frequencies of Ni resistance than Cu were found in five strains: *Streptomyces labedae* C1, *Streptomyces vinaceusdrappus* C2, *Streptomyces huasconensis* C8, *Streptomyces griseoincarnatus* C9, and *Streptomyces coelicoflavus* C10. However, *Streptomyces thermocarboxydus* C3 observed higher resistance to Cu than Ni. In addition, *Streptomyces harenosi* C4 was nickel-resistant only, *Streptomyces griseoflavus* C7 was just copper-resistant, and no resistance to the two tested heavy metals was found in the *Streptomyces griseorubens* C5 isolate.

Among various concentrations of two metals, MTL of *S. labedae* C1, *S. vinaceusdrappus* C2, *S. ursincola* C6, and *S. griseoincarnatus* C9 was 300 mg/L of Ni. However, *S. coelicoflavus* C10 and *S. huasconensis* C8 could survive up to 250 and 200 mg/L of Ni, respectively. The isolates *S. thermocarboxydus* C3 and *S. harenosi* C4 recorded MTLs of Ni at 50 mg/L. Based on the results of Cu, only *S. ursincola* C6 could withstand Cu concentrations of 300 mg/L, and *S. vinaceusdrappus* C2 and *S. huasconensis* C8 had MTL values of 200 and 150 mg/L, respectively. Four strains, *S. labedae* C1, *S. thermocarboxydus* C3, *S. griseoincarnatus* C9, and *S. coelicoflavus* C10, tolerated only up to 100 mg/L. Whilst *S. griseoflavus* C7 had an MTL of 50 mg/L.

Ni metal not only affected bacterial growth quality but also influenced colony pigmentation in some isolates. The media incorporated Ni made changes in the color of some metal-tolerant strains growing on plates. Figure 5 illustrates the effects of selected metal concentrations on bacterial growth and colony color.

The percentage of bacterial strains' tolerance to both metals was similar (80%) at a concentration of 50 mg/L. Then, at a concentration of 100 mg/L, this ratio decreased to 70% for Cu-resistant isolates and to 60% for Ni resistance. Compared with the higher concentrations tested, the Ni-resistant average declined gradually, with 40% at the highest concentration (300 mg/L). However, the percentage of resistant bacteria to Cu decreased significantly while the concentration increased, and at 300 mg/L, it was about 10% (Figure 6).

Antibacterial Activity of Isolates

In the course of screening for antibacterial properties, the selected bacterial strains were tested against pathogens: *S. hominis* and *E. coli*. Antibacterial activity of grown isolates in media without added metals or with 50 mg/L Cu or Ni was assessed by measuring inhibition zone diameters (IZDs) around the disk and calculating the mean ± SD of replicates to determine significance ($P < 0.05$).

The findings revealed that six isolates (growing on media without any metal) inhibited the growth of the Gram-positive bacterium (*S. hominis*) only, with a higher potency of *Streptomyces vinaceusdrappus* C2 and *Streptomyces huasconensis* C8 to produce antibiotic substances than other isolates (Figure 7 and Table 3). As can be seen, *S. vinaceusdrappus* C2 and *S. huasconensis* C8 recorded inhibition zones of 21.5 ± 0.5 and 21 ± 0.63 mm, respectively. However, *Streptomyces griseoflavus* C7 and *Streptomyces griseoincarnatus* C9 exhibited almost similar activity against the target *S. hominis*, with a zone of inhibition diameter measuring 13.5 ± 0.13 and 13 ± 0.1 mm, respectively, followed by *Streptomyces griseorubens* C5 (12 ± 0.15 mm) and *Streptomyces coelicoflavus* C10 (11 ± 0.59 mm). Figure 8 illustrates the antibacterial potential of some actinomycetes against two pathogens.

Based on the results of the first experiment to determine antibacterial activity, five actinomycetes with both metal resistance and antibacterial properties were selected. Notably, the diameter of the clear inhibition zone increased for all tested *Streptomyces* species in the presence of Cu or Ni (50 mg/L) in the growth medium. Disc controls (metal-supplemented media without isolates) did not show any inhibition to *S. hominis* growth (Table 3). The inhibition zones reached maximum values (27.5 ± 0.5 mm) in the presence of Cu for *S. vinaceusdrappus* C2 and 27 ± 0.23 mm in

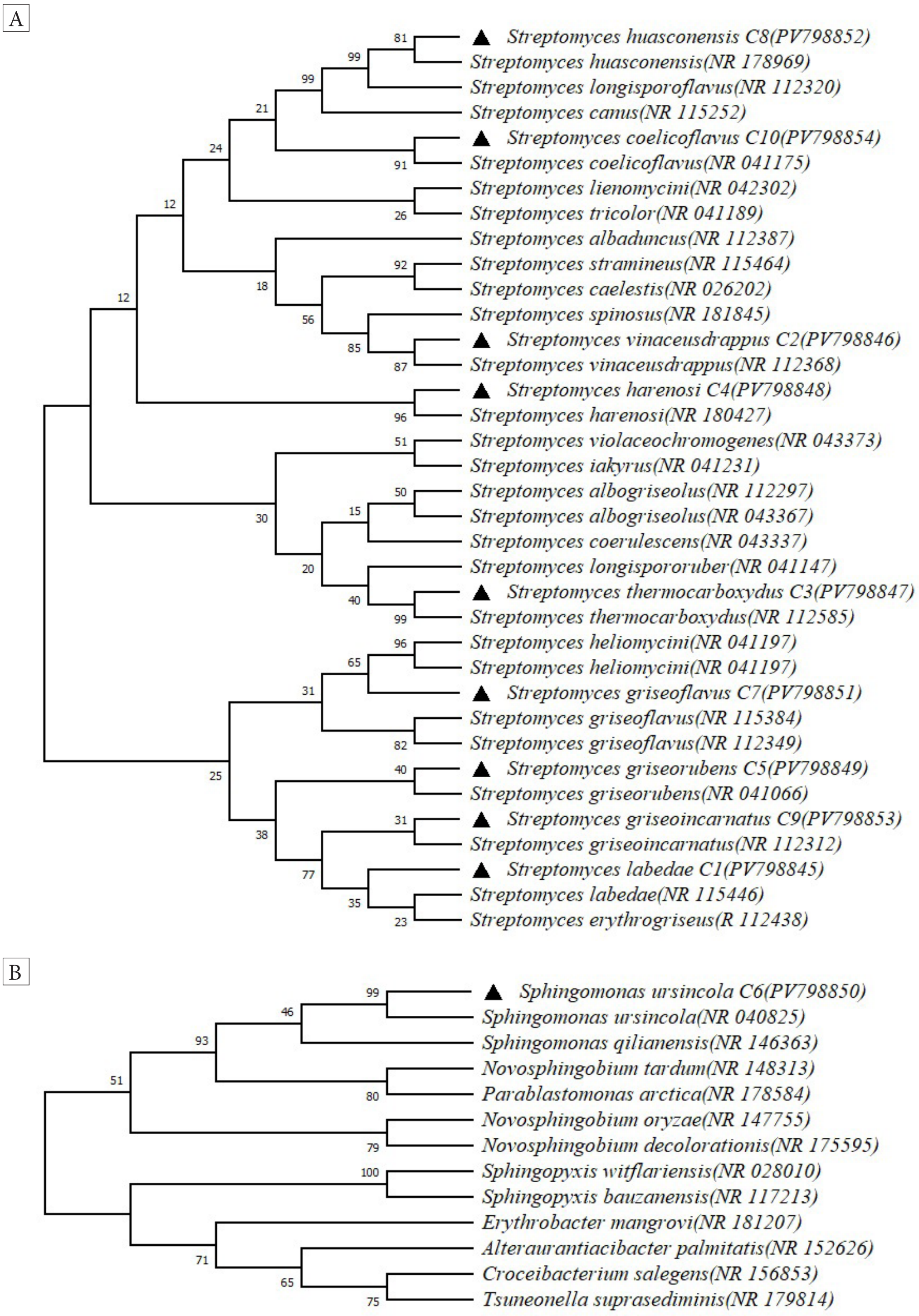

Fig. 3 **Phylogenetic analysis of cave isolates identified as (A) *Streptomyces* spp. and (B) *Sphingomonas ursincola*. Neighbor-joining phylogenetic tree showing the evolutionary relationship of the selected isolates based on 16S rRNA sequence alignments.**

the presence of Ni for *S. huasconensis* C8, indicating that the presence of metals enhanced the antimicrobial activities of the tested actinomycete isolates.

Based on the results of this study, the isolate *Sphingomonas ursincola* C6, which was the most tolerant to the selected metal concentrations, did not exhibit any antibacterial properties against the tested pathogens. In addition, although *S. griseorubens* C5 did not tolerate or grow in media supplemented with the test metals at all, it could inhibit the growth of *S. hominis*. These two examples indicate that there is no correlation between the isolates' ability to resist heavy metals and their potential to produce antibacterial products. Nevertheless, *S. vinaceusdrappus* C2, which had the greatest antibacterial activity among all isolates, was also Cu- and Ni-resistant.

Among all isolates, four heavy metal-resistant bacteria also produced antibiotics, as shown in Figure 9. These two capabilities likely provide a competitive advantage for cave bacteria, enabling them to survive in this extreme environment. In this sense, the following strains (*S. vinaceusdrappus*

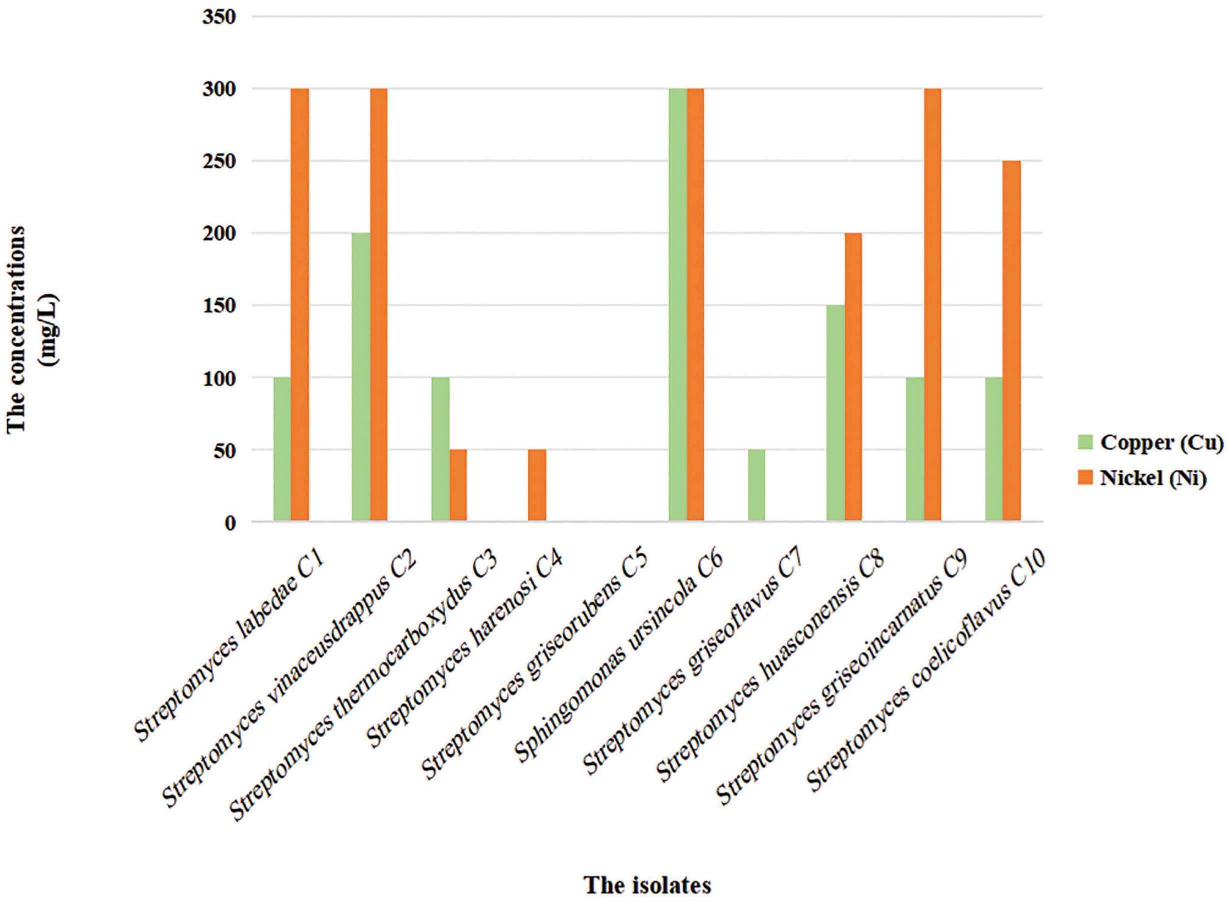

Fig. 4 **Maximum tolerance level (MTL) values of copper (Cu) and nickel (Ni) tolerance in bacterial isolates.**

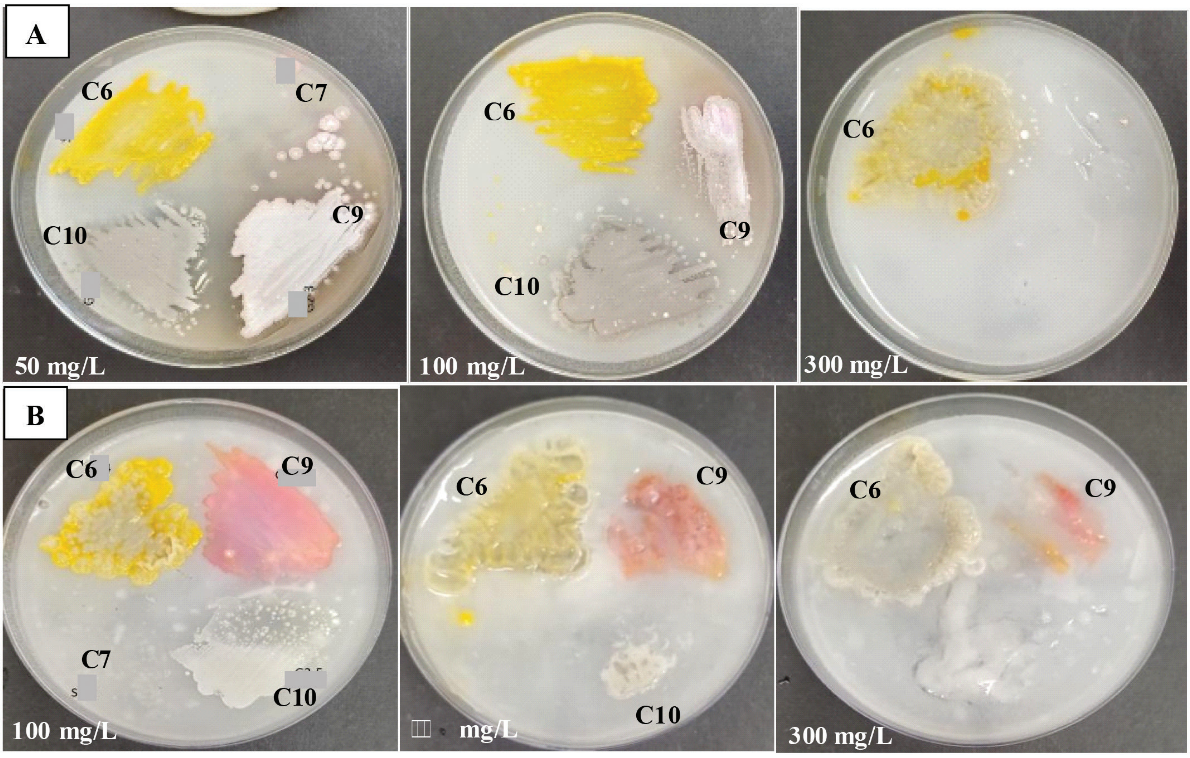

Fig. 5 **Growth of some bacterial isolates on starch nitrate agar amended with varying concentrations of a heavy metal, (A): Copper (Cu) and (B): Nickel (Ni).**

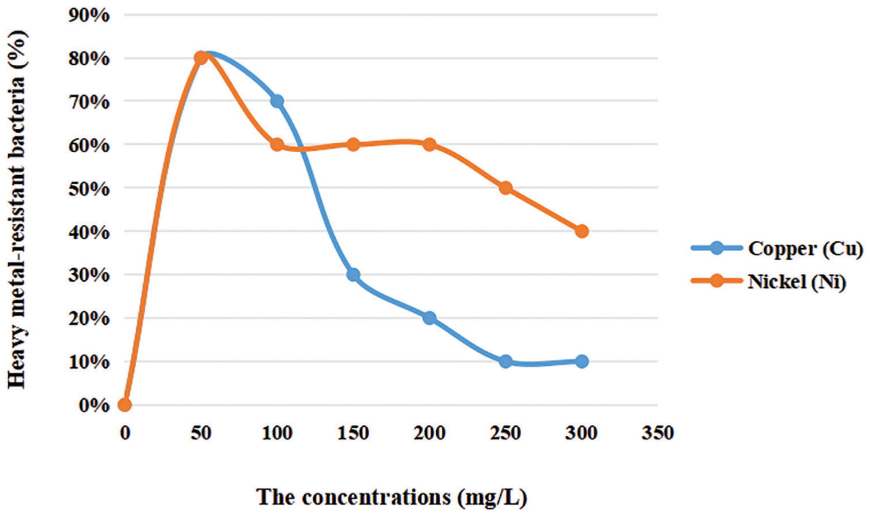

Fig. 6 **Percentage of bacteria resistance to various concentrations of copper (Cu) and nickel (Ni).**

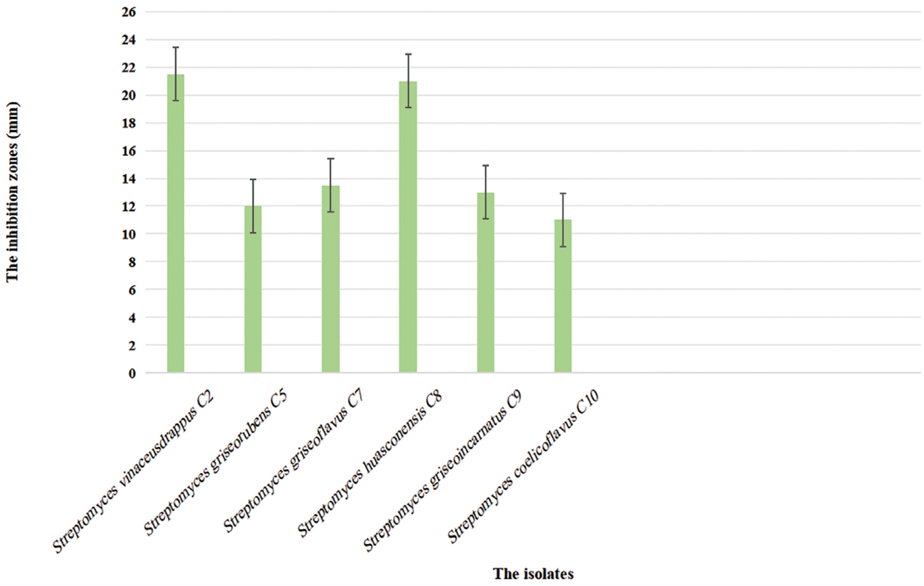

Fig. 7 **Mean ± SD of inhibition zone diameters against *Staphylococcus hominis* of isolated actinomycetes (metal-free medium).**

Table 3. **Inhibition zones produced by the tested actinomycetes in the absence or presence of 50 mg/L of copper (Cu) or nickel (Ni) against *Staphylococcus hominis***

The tested agent	IZD (metal-free medium)	IZD (in the presence of Cu)	IZD (in the presence of Ni)
Cu (50 mg/L, control)	ND	0.0	ND
Ni (50 mg/L, control)	ND	ND	0.0
Streptomyces vinaceusdrappus C2	21.5 ± 0.50	27.5 ± 0.50*	25.5 ± 0.01*
Streptomyces griseorubens C5	12.0 ± 0.15	ND	ND
Streptomyces griseoflavus C7	13.5 ± 0.13	14.5 ± 0.11*	ND
Streptomyces huasconensis C8	21.0 ± 0.63	24.0 ± 0.22*	27.0 ± 0.23*
Streptomyces griseoincarnatus C9	13.0 ± 0.10	17.0 ± 0.12*	15.0 ± 0.20*
Streptomyces coelicoflavus C10	11.0 ± 0.59	14.0 ± 0.19*	15.0 ± 0.15*

The data were expressed as the mean value ± SD, *Significant results at $P < 0.05$. IZD: Inhibition zone diameter (mm), ND: Not done.

C2, *S. huasconensis* C8, *S. griseoincarnatus* C9, and *S. coelicoflavus* C10) could be selected as promising sources for bioremediation in metal-contaminated environments and for the isolation of novel antimicrobial metabolites with potential applications in pharmaceutical sectors.

Discussion

Caves present distinctive environments where light, temperature, and nutrients are in short supply.[27] Within these unique settings, the cave microbiome is a potential source of new, valuable commercial compounds.[11] Research conducted in various caves globally has revealed that actinobacteria, particularly the genus *Streptomyces*, dominate these extreme ecosystems.[28] Long et al.[29] isolated 239 actinomycetes from Shuanghe Cave in China, and *Streptomyces* was the dominant genus, representing 52% of the isolates. Similarly, the results of this research showed that of the 10 bacterial species isolated from Umm Jirsan Cave, nine were identified as various species under the *Streptomyces* genus. Only one isolate, *Sphingomonas ursincola* C6, was determined to belong to Proteobacteria.

More than 70% of antibiotics originate from actinobacteria, especially the *Streptomyces* species.[28] Cave actinobacteria have shown the ability to inhibit various pathogenic bacteria, including both Gram-positive and Gram-negative strains such as *Salmonella* and *Escherichia coli*, even those causing severe illnesses such as vancomycin-resistant *Enterococcus* (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), and Shiga-toxin-producing *E. coli* (STEC).[30] Iquebal et al.[31] successfully isolated 235 bacterial strains from Pukzing Cave in India, with actinomycetes standing out as the most prevalent group. Notably, about 20.4% of these strains showcased impressive broad-spectrum antimicrobial properties against various pathogens, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Candida albicans*.

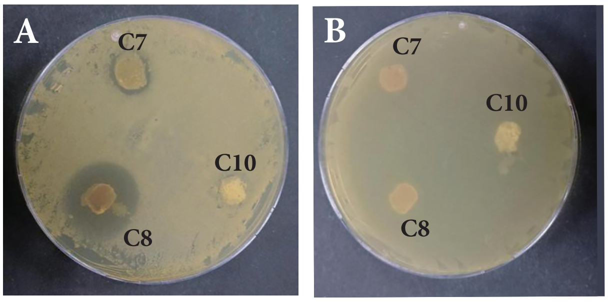

Fig. 8 **Screening of the antibacterial properties of some isolates (metal-free medium) against selected pathogens, (A): *Staphylococcus hominis* and (B): *Escherichia coli*.**

The rise of drug resistance in infections caused by *Staphylococcus hominis*[32] and *Escherichia coli*[33] limits treatment options and remains a challenge. Our results confirmed the ability of isolated actinomycetes to produce antibiotics specifically against *S. hominis*, with six isolates showing inhibition zones measuring from 11.0 ± 0.59 to 21.5 ± 0.5 mm. Interestingly, Pipite et al.[12] noted that cave actinomycetes, which exhibited growth inhibition of some pathogens, were capable of producing distinctive bioactive compounds.

Heavy metals, including copper (Cu) and nickel (Ni), have become pervasive environmental pollutants due to industrial activities, agricultural practices, and mining, leading to significant effects on actinomycete growth and antibiotic production. Their impact on microbial communities, particularly in terms of antibiotic production, is an area of growing research interest, as their influence on antibiotic biosynthesis is not well-known.[34]

In the current study, 70% of the isolated bacteria from Umm Jirsan Cave displayed resistance to Cu and Ni to various degrees. The potent isolate, *Sphingomonas ursincola* C6, a non-actinomycete, Gram-negative bacterium, was able to grow on agar media containing the highest tested concentration of Cu or Ni (300 mg/L). In a previous study conducted in Hampoeil Cave (Iran), the isolated actinomycetes were screened for their biological activities. About 25.3% of the isolates had antimicrobial activity, and 26.32% were resistant to nickel (Ni), cadmium (Cd), and copper (Cu).[18]

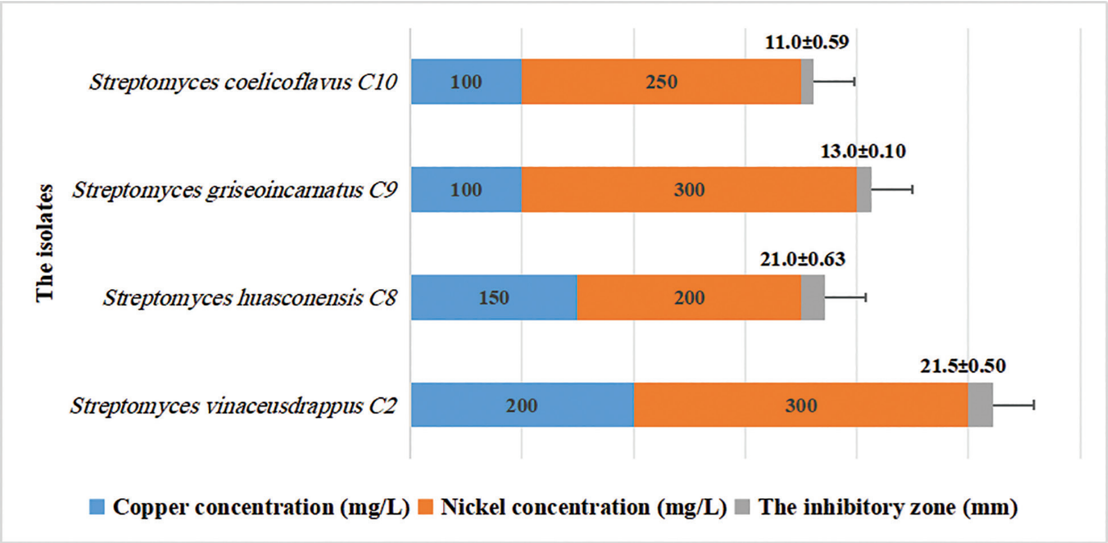

Fig. 9 **Four strains produced antibacterial compounds against *Staphylococcus hominis* (measured as inhibition zones) and were also tolerant to copper (Cu) and nickel (Ni) (the highest concentration of resistance was shown).**

The variations in bacteria's response to heavy metals may result from differences in cell wall composition or in resistance strategies. There are three main mechanisms by which bacteria resist metal toxicity: efflux pump systems, sequestration of metals both inside and outside the cell, and enzymatic transformation of metals into less harmful forms. The key to bacterial resistance to heavy metals lies in the regulation of genes that govern functions of efflux pumps, as well as those involved in metal uptake, sequestration, and detoxification.[13]

It is well known that copper (Cu) is an essential trace element required for various biological processes; however, at elevated concentrations, it can be toxic. Studies have shown that Cu can inhibit the growth of several microorganisms, which may lead to promoting the synthesis of antibiotics. Some reports indicated that sub-inhibitory concentrations of Cu can stimulate antibiotic production. For instance, low concentrations of Cu can enhance the production of certain antibiotics such as erythromycin in *Saccharopolyspora erythraea*.[35] Similarly, Yin et al.[36] reported that Cu significantly reduced antibiotic synthesis in *Streptomyces* species, while at low concentrations it induced production. This phenomenon may be linked to stress response mechanisms triggered by low levels of metal ions, which can activate secondary metabolite pathways. On the other hand, studies on nickel (Ni) were fewer than those on copper (Cu), and Ni also plays a vital role in microbial metabolism, but poses toxicity at higher levels. Research has demonstrated that Ni can induce stress responses in bacteria, leading to alterations in metabolic pathways responsible for antibiotic production.[37]

Our investigation showed that Cu and Ni at the lowest concentration (50 mg/L) increased the inhibition zone diameter against *S. hominis* of all tested actinomycetes, emphasizing the influential role of low metal concentrations in antibiotic production. In the presence of 50 mg/L of Cu in the medium, the highest inhibition zone recorded was 27.5 ± 0.5 mm for *Streptomyces vinaceusdrappus* C2, and *Streptomyces huasconensis* C8 exhibited a clear inhibition zone of 27 ± 0.23 mm in the medium with Ni.

The observed responses of microbial populations to heavy metals, particularly regarding antibiotic production, can be attributed to various mechanisms. Heavy metals can stimulate oxidative stress, leading to the activation of response genes, which could either promote or inhibit biosynthesis of secondary metabolites, including antibiotics. The production of reactive oxygen species (ROS) can damage cellular components but may also stimulate signaling pathways associated with antibiotic production. Moreover, heavy metals can alter membrane permeability and affect nutrient uptake, which is critical for antibiotic production. Thus, disrupting ion gradients across the membrane, which can impair nutrient assimilation needed for metabolic processes, leads to antibiotic synthesis.[38]

Conclusion

Cave actinomycetes can play a pioneering role in current environmental and medical issues by detoxifying metals and producing distinctive secondary metabolites on a large scale. In this study, out of ten bacterial species, nine actinomycetes were successfully isolated from the cave habitat. Among them, four actinomycetes exhibited both good antibacterial activity and metal tolerance, certainly worthy of further investigation. Interestingly, the findings showed that although heavy metals are toxic, they may serve as stimulants for antibiotic synthesis in specific contexts. More studies are needed to delineate the metabolic pathways influenced by these heavy metals to harness this knowledge for biotechnological applications for antibiotic production.

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Conflict of Interest

None.

References

1. Briffa, J., Sinagra, E. and Blundell, R. (2020). Heavy metal pollution in the environment and their toxicological effects on humans. Heliyon, 6(9): e04691.
2. Nkwunonwo, U.C., Odika, P.O. and Onyia, N.I. (2020). A review of the health implications of heavy metals in food chain in Nigeria. The Scientific World Journal, 2020(1): 6594109.
3. Xu, W., Jin, Y. and Zeng, G. (2024). Introduction of heavy metals contamination in the water and soil: a review on source, toxicity and remediation methods. Green Chemistry Letters and Reviews, 17(1).
4. Ayilara, M.S. and Babalola, O.O. (2023). Bioremediation of environmental wastes: the role of microorganisms. Frontiers in Agronomy, 5: 1183691.
5. Sable, H., Singh, V., Kumar, V., Roy, A., Pandit, S., Kaur, K. et al. (2024). Toxicological and bioremediation profiling of nonessential heavy metals (mercury, chromium, cadmium, aluminium) and their impact on human health: a review. Toxicologie Analytique et Clinique, 36(3): 205–234.
6. WHO (2023). Antimicrobial resistance. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance.
7. Majhi, S. (2021). Applications of Yamaguchi method to esterification and macrolactonization in total synthesis of bioactive natural products. ChemistrySelect, 6(17): 4178–4206.
8. Mancuso, G., Midiri, A., Gerace, E. and Biondo, C. (2021). Bacterial antibiotic resistance: the most critical pathogens. Pathogens, 10(10): 1310.
9. O'Neill, J. (2016). Tackling drug-resistant infections globally: final report and recommendations. The review on antimicrobial resistance. London.
10. Walsh, T.R., Gales, A.C., Laxminarayan, R. and Dodd, P.C. (2023). Antimicrobial resistance: addressing a global threat to humanity. PLoS Medicine, 20(7): e1004264.
11. Khatri, A., Kumar, K. and Thakur, I.S. (2025). Microbiome of caves for bioprospecting: a critical review. Systems Microbiology and Biomanufacturing, 5: 550–566.
12. Pipite, A., Lockhart, P.J., McLenachan, P.A., Christi, K., Kumar, D., Prasad, S. and Subramani, R. (2022). Isolation, antibacterial screening, and identification of bioactive cave dwelling bacteria in Fiji. Frontiers in Microbiology, 13: 1012867.
13. Nnaji, N.D., Anyanwu, C.U., Miri, T. and Onyeaka, H. (2024). Mechanisms of heavy metal tolerance in bacteria: a review. Sustainability, 16(24): 11124.
14. Farda, B., Djebaili, R., Vaccarelli, I., Del Gallo, M. and Pellegrini, M. (2022). Actinomycetes from caves: an overview of their diversity, biotechnological properties, and insights for their use in soil environments. Microorganisms, 10(2): 453.

15. Pint, J. (2009). Umm Jirsan: Arabia's longest lava-tube system. 15th International Congress of Speleology, 714–717.
16. Németh, K. and Moufti, M.R. (2023). Lava flow hazard and its implication in geopark development for the active Harrat Khaybar intracontinental monogenetic volcanic field, Saudi Arabia. Land, 12(3): 705.
17. Shirling, E.B. and Gottlieb, D. (1966) Methods for characterization of *Streptomyces* species. International Journal of Systematic Bacteriology, 16: 313–340.
18. Hamedi, J., Kafshnouchi, M. and Ranjbaran, M. (2019). A study on actinobacterial diversity of Hampoeil Cave and screening of their biological activities. Saudi Journal of Biological Sciences, 26(7): 1587–1595.
19. Uzel, A., Hameş kocabaş, E.E. and Bedir, E. (2011). Prevalence of *Thermoactinomyces thalpophilus* and *T. sacchari* strains with biotechnological potential at hot springs and soils from West Anatolia in Turkey. Turkish Journal of Biology, 35(2): 195–202.
20. Yáñez, M.A., Catalán, V., Apráiz, D., Figueras, M.J. and Martínez-Murcia, A.J. (2003). Phylogenetic analysis of members of the genus *Aeromonas* based on gyrB gene sequences. International Journal of Systematic and Evolutionary Microbiology, 53(3): 875–883.
21. Momtaz, H. and Yadollahi, S. (2013). Molecular characterization of *Listeria monocytogenes* isolated from fresh seafood samples in Iran. Diagnostic Pathology, 8: 1–6.
22. Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990). Basic local alignment search tool. Journal of Molecular Biology, 215(3): 403–410.
23. Amasha, R.H., Alzahrani, N. and Aly, M.M. (2020). Effect of nickel on growth of some actinomycetes isolated from three caves in Saudi Arabia. La Prensa Medica Argentina, S2:022.
24. Radhakrishnan, M., Gopikrishnan, V., Balaji, S., Balagurunathan, R. and Vanaja K. (2014). Bioprospecting of actinomycetes from certain less explored ecosystems active against *Mycobacterium tuberculosis* and other non-mycobacterial pathogens. International Scholarly Research Notices, 2014: 812974.
25. Janaki, T., Nayak, B.K. and Ganesan, T. (2016). Antibacterial activity of soil actinomycetes from the mangrove *Avicennia marina*. Journal of Pharmacognosy and Phytochemistry, 5(1): 267.
26. Thampi, N. and Kumar, S.N. (2015). Evaluation of antimicrobial and free radical scavenging potentials of three valuable medicinal plants-*Hyptis suaveolens*, *Spathodea campanulata* and *Shorea robusta*. Journal of Chemical and Pharmaceutical Research, 7(7): 609–615.
27. Cheeptham, N. (2013). Advances and challenges in studying cave microbial diversity. In: Cheeptham, N. (eds.), Cave microbiomes: a novel resource for drug discovery. Springer Briefs in Microbiology, vol 1. Springer, New York, NY.
28. Mpakosi, A. and Mironidou-Tzouveleki, M. (2023). The cave ecosystem in the research of new antibiotic discovery and development. Environmental Sciences Proceedings, 26(1): 116.
29. Long, Y., Jiang, J., Hu, X., Zhou, J., Hu, J. and Zhou, S. (2019). Actinobacterial community in Shuanghe Cave using culture-dependent and -independent approaches. World Journal of Microbiology and Biotechnology, 35(10): 153.
30. Kosznik-Kwaśnicka, K., Golec, P., Jaroszewicz, W., Lubomska, D. and Piechowicz, L. (2022). Into the unknown: microbial communities in caves, their role, and potential use. Microorganisms, 10(2): 222.
31. Iquebal, M.A., Passari, A.K., Jagannadham, J., Ahmad, F., Leo, V.V., Singh, G. et al. (2021). Microbiome of Pukzing Cave in India shows high antimicrobial activity against plant and animal pathogens. Genomics, 113(6): 4098–4108.
32. Szemraj, M., Glajzner, P., Olszowiec, K. and Sienkiewicz, M. (2025). The prevalence of multidrug resistance in *Staphylococcus hominis* isolated from clinical materials. Scientific Reports, 15(1): 414.
33. Ganjo, A.R. (2022). Extended-spectrum β-lactamases and AMPC production among uropathogenic isolates of *Escherichia coli* and antibiogram pattern. ARO-The Scientific Journal of Koya University, 10(1): 98–103.
34. Ueda, K., Tomaru, Y., Endoh, K. and Beppu, T. (1997). Stimulatory effect of copper on antibiotic production and morphological differentiation in *Streptomyces tanashiensis*. The Journal of Antibiotics (Tokyo), 50(8): 693–5.
35. Qiao, L., Li, X., Ke, X. et al. (2020). A two-component system gene *SACE_0101* regulates copper homeostasis in *Saccharopolyspora erythraea*. Bioresour. Bioprocess**,** 7: 12.
36. Yin, Y., Gu, J., Wang, X., Song, W., Zhang, K., Sun, W., Zhang, X., Zhang, Y. and Li, H. (2017). Effects of copper addition on copper resistance, antibiotic resistance genes, and *intl1* during swine manure composting. Frontiers in Microbiology, 8: 344.
37. Abbas, A.S. and Edwards, C. (1990). Effects of metals on *Streptomyces coelicolor* growth and actinorhodin production. Applied and Environmental Microbiology, 56(3): 675–80.
38. Sánchez-Corona, C.G., Gonzalez-Avila, L.U., Hernández-Cortez, C., Rojas-Vargas, J., Castro-Escarpulli, G. and Castelán-Sánchez, H.G. (2025). Impact of heavy metal and resistance genes on antimicrobial resistance: ecological and public health implications. Genes (Basel), 16(6): 625.