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Isolation and characterization of native strains from lithium-containing mining tailings

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Abstract

Lithium-rich mining tailings create extreme and poorly explored environments that can serve as reservoirs for microorganisms with unique biotechnological potential. In this study, native microorganisms were isolated and molecularly characterized from the tailings of the Nochebuena mine, Zacatecas, Mexico. Six pure cultures were obtained using serial dilution and cross-streaking methods. Lithium tolerance was assessed through minimum inhibitory concentration (MIC) assays, revealing resistance levels between 5,000 and 20,000 ppm LiCl. Molecular characterization involved DNA extraction, amplification, purification, and restriction digestion with the HhaI enzyme, followed by sequencing. The isolates were identified as Stutzerimonas chloritidismutans, Microbacterium paraoxydans, Stenotrophomonas maltophilia, Bacillus cereus, and Bacillus ludwigii. Phylogenetic analysis was performed to resolve their evolutionary relationships. These findings highlight the untapped microbial diversity in lithium-rich mining residues and its promise for innovative applications in biohydrometallurgy.

Keywords: Lithium, Mining tailings, Biohydrometallurgy, Phylogenetic analysis.

1. Introduction

Lithium, named after the Greek word *lithos* (stone), is a silvery-white metal recognized for its low density, high reactivity, and low melting point. These properties have made it indispensable for the manufacture of high-energy-density batteries, which power electronic devices, electric vehicles, and large-scale energy storage systems for renewable sources such as solar and wind [1]. As global demand for sustainable alternatives to fossil fuels grows, lithium has emerged as a cornerstone of the energy transition, often referred to as "white gold" [2].

Despite its strategic importance, lithium extraction presents significant environmental challenges. Brine mining, for example, lowers groundwater levels, disrupting fragile ecosystems, migratory bird populations, and local communities [3]. In contrast, hard-rock extraction involves energy-intensive chemical processing, consuming vast amounts of water and generating toxic waste, which compounds ecological damage.

To mitigate these impacts, biotechnology offers more sustainable approaches to lithium recovery. Biohydrometallurgy—particularly bioleaching—relies on the ability of native microorganisms to mobilize metals from ores and tailings [4]. Bacteria and fungi adapted to extreme environments can dissolve mineral compounds through their metabolic activity, reducing reliance on harsh chemicals and minimizing water use.

Central to this strategy is the identification and characterization of microorganisms capable of tolerating and transforming lithium. Isolation of indigenous strains from lithium-rich mine residues provides the foundation for such efforts. These strains can be investigated using molecular techniques, including DNA extraction and PCR amplification, to uncover genes and proteins linked to lithium resistance and solubilization [5,6]. Enzymatic assays further illuminate the metabolic pathways underpinning microorganism–mineral interactions.

Bioinformatics strengthens this approach by enabling large-scale genomic and proteomic analysis of the isolates [6,7]. Computational tools help identify key genes and metabolic networks involved in lithium mobilization, while phylogenetic reconstructions and functional protein

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analyses aid in selecting strains with superior bioleaching potential. Together, these methods not only enhance the efficiency of microbial lithium recovery but also open the door to designing tailored microbial consortia optimized for specific mining environments.

2. Materials and methods

A sample of tailings was received from the Noche Buena mine, located in the municipality of Melchor Ocampo, Zacatecas, Mexico (Figure 1).

2.1. Characterization of the tailings

The characterization of the tailings from the Noche Buena mine was carried out at SGS México (Société Générale de Surveillance) in the geochemical and metallurgical analysis division. This multinational company, located in Durango, Durango (México), specializes in services for the mining and exploration sector. The analysis included a multielement characterization of 34 elements through digestion with aqua regia [8].

2.2. Isolation and morphological characterization of microorganisms

A sample of tailings from the mine was collected for the isolation of native microorganisms. Luria-Bertani (LB), 9K, and API media were selected to cover a broad range of microorganisms. Each medium was strategically chosen: LB as a nutrient-rich medium with an organic carbon source, and 9K and API as selective media for specific microorganisms, including autotrophic and lithotrophic bacteria capable of surviving under extreme conditions. Table 1 details the composition of each culture medium used [6, 7]. For the enrichment of microorganisms present in the mine tailings, samples were added at a 10% (w/v) ratio to 250 mL of each culture medium, preparing duplicates for each experimental condition. The samples were incubated at 30 °C and 180 rpm for 5 days in LB medium, 10 days in API medium, and 21 days in 9K medium. After the enrichment process, each culture was serially diluted to 10^{-7} and re-inoculated onto the respective solid medium. Colonies obtained after 48 hours of incubation at 30 °C were selected based on their morphology, ensuring that only individual isolated colonies were chosen. To obtain pure cultures, at least five streaking steps were performed. Subsequently, staining procedures were conducted for microorganism characterization, including a Gram stain [9]. In parallel with the enrichment process, the redox potential (ORP) and pH of each culture medium were recorded over 30 days to gather detailed information on the interactions between the medium and the tailings.

2.3. Microbial growth kinetics

The characteristic growth curve without LiCl (control) was determined using UV-Vis spectrophotometry at 600 nm (Thermo Scientific G10S UV-Vis BIO). The strains, identified as PRLB, PBLB, AAPI, BAPI, and *E. coli BL21* (control), were inoculated into liquid medium for 12 hours. Subsequently, serial dilutions were performed to adjust the initial concentration to 0.01 OD in fresh, non-inoculated medium. The experiment was conducted in triplicate, maintaining controlled conditions of temperature (30 °C) and agitation (180 rpm). Optical density measurements were recorded at 2-hour intervals over a period of 48 hours to assess the growth of each strain [10]. Using the determined growth curve, the doubling time (Td) was calculated in hours using Equation 1, where μ represents the specific growth rate (1/h), obtained from the exponential phase of the growth curve.

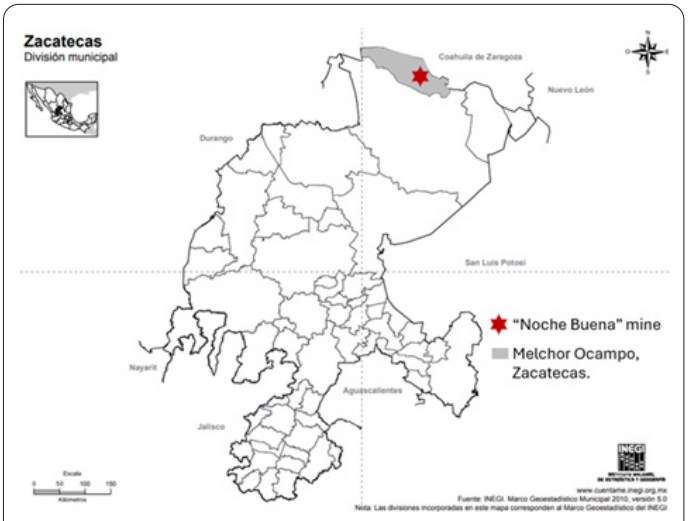

Fig. 1. Location of the Noche Buena mine operated by Fresnillo PLC.

$$Td = \frac{ln2}{\mu} \quad (1)$$

2.4. Minimum inhibitory concentration tests

The evaluation of the isolated strains was carried out following the technique described by Moreno and Albarracín in 2012 [11], exposing them to varying concentrations of LiCl to analyze their response to this compound. Each strain was inoculated into a liquid medium supplemented with LiCl at concentrations of 10,000 ppm, 15,000 ppm, and 20,000 ppm. Growth monitoring was performed over 48 hours by measuring optical density every 2 hours [10, 12].

2.5. Molecular characterization of isolated microorganisms

2.5.1 Bacterial DNA extraction

For DNA extraction, the strains were cultured in LB liquid medium at 180 rpm and 30 °C for 24 hours. Sub-

Table 1. Composition of the culture medium used for the isolation of native microorganisms.

LB	API	9K
Peptone = 10 g/L	Yeast extract = 1 g/L	$(NH_4)_2SO_4$ = 3 g/L
Yeast extract = 5 g/L	$C_6H_8O_6$ = 0.1 g/L	KCl = 0.1 g/L
NaCl =10 g/L	C_3H5NaO_3 = 4 mL/L	K_2HPO_4 = 0.5 g/L
	$MgSO_4 \cdot 7H_2O$ = 0.2 g/L	$MgSO_4 \cdot 7H_2O$ = 0.5 g/L
	K_2HPO_4 = 0.01 g/L	$Ca(NO_3)_2$ = 0.01 g/L
	$(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ = 0.1 g/L	$FeSO_4$ = 14 g/L
	NaCl = 10 g/L	

sequently, 1 mL of the culture was centrifuged at 13,000 rpm for 2 min, and the cell pellet obtained was resuspended in 300 µL of lysis buffer composed of EDTA (0.05 M), NaCl (0.1 M) and adjusted to pH 7.5, by gentle vortexing for 30 sec. Next, 100 µL of lysozyme solution (10 mg/mL) and 30 µL of 20% (w/v) SDS were added. The mixture was incubated at 37 °C for 5 min to promote cell lysis. The quality of DNA extraction was verified by 1% agarose gel electrophoresis stained with ethidium bromide. Finally, DNA was purified using a phenol-chloroform-isoamyl alcohol mixture (25:24:1, v/v/v/v). This procedure was performed in triplicate to ensure reproducibility and quality of the extracted material. [10, 12, 13]

2.5.2. 16S rRNA amplification

After confirming the integrity of the bacterial DNA through agarose gel electrophoresis, the amplification of the 16S rRNA gene was performed following the specified reaction mixture for a total volume of 25 µL, with primers 27f and 1492r (5'-AGAGTTTGATCCTGGCTCAG-3' y 5'- GGCTACCTTGTTACGACTT -3') [12, 14].

The reaction mixture consisted of 0.5 µL of dNTPs (10 mM), 5 µL of GoTaq 5x buffer, 0.5 µL of each primer, 0.25 µL of DNA polymerase, 1.5 µL of $MgCl_2$, 14.75 µL of nuclease-free water, and 2 µL of DNA sample. The mixture was prepared in a container on ice. Following a temperature gradient for primer Tm selection in a thermal cycler (T100-BioRad, Irvine, CA, USA), the PCR conditions were as follows: an initial denaturation step for 3 minutes at 95 °C, followed by 30 cycles of denaturation for 30 seconds at 70 °C, an alignment step for 30 seconds at 55 °C (the optimal band pattern obtained in the temperature gradient test), and an extension step for 1 minute at 72 °C, with a final extension of 5 minutes at 72 °C. Positive PCR products were evaluated on a 1% agarose gel stained with ethidium bromide (0.3 µg/mL), using a 1 kb molecular marker for size comparison and a 5x green sample loading buffer.

2.6. DNA Restriction

For the molecular characterization of the isolated strains, an enzymatic restriction protocol was employed using *HhaI* [15]. The digestion reaction was prepared in sterile tubes with 3 µL of purified PCR product, 0.5 µL of *HhaI*, 1 µL of Buffer C, and 5.5 µL of nuclease-free water. The mixture was properly homogenized and incubated at 37 °C for 3 hours in a Felisa FE-133A incubator (Feligneo S.A. de C. V., Jalisco, Mexico), ensuring maximum enzyme activity and the generation of specific cuts in the DNA sequences. Subsequently, electrophoresis was performed on a 2% agarose gel prepared with 0.6 g of agarose in 30 mL of 1% TAE buffer and stained with ethidium bromide. The solution was poured into a tray and allowed to solidify for 30 minutes. A total of 10 µL of the enzymatic digestion and 5 µL of Green GoTaq Flexi Buffer loading dye were loaded, along with a 100 bp marker for fragment size estimation.

2.7. Bioinformatic analysis

After purifying the PCR product following the specifications of the purification kit, the amplicons were sequenced using the Sanger method at the Institute of Biotechnology [16], UNAM (Cuernavaca, Morelos, Mexico). The resulting sequences were processed and edited in BioEdit (2020) to ensure quality, generating a consensus sequence. This consensus was then used to identify homologous sequences through the BLAST tool provided by the National Center for Biotechnology Information (NCBI) [17].

Phylogenetic trees were constructed in MEGA 11 using the Maximum Likelihood method with the Tamura-Nei model [18]. The UPGMA method was applied, and 1,000 bootstrap replications were performed to evaluate the robustness of the internal branches [19]. The resulting trees display the highest log-likelihood values, indicating the percentage clustering of taxa at each node. The initial tree search was conducted heuristically using the Neighbor-Joining and BioNJ algorithms on a pairwise distance matrix estimated with the Tamura-Nei model [20], selecting the topology with the highest likelihood. The final dataset comprised 1,912 positions, and all evolutionary analyses were performed in MEGA 11 [20].

3. Results

3.1. Characterization of mining tailings

Table 2. Results of the multielement analysis by aqua regia digestion of the tailings obtained from the Noche Buena Mine, highlighting the most relevant elements for the study, respectively.

The characterization results of the Noche Buena mine tailings (Table 2) reveal significant concentrations of key elements relevant to this study. This site is one of the three main lithium exploration deposits in the country, according to studies conducted by Litiomex [21]. Preliminary analyses indicate significant potential, with estimated resources of 8 million tons of lithium equivalent. The selection of this deposit was based on its strategic relevance and the interest in leveraging lithium through bioleaching techniques, including the isolation of native microorganisms capable of optimizing metal recovery [1]. Notably, lithium (Li: 8 ppm) is present at levels that position it as a viable candidate for bioleaching-based extraction. Regar-

Table 2. Results of the multielement analysis by aqua regia digestion of the tailings obtained from the Noche Buena Mine, highlighting the most relevant elements for the study, respectively.

Analyte	mg/Kg (ppm)
Al	2090
As	>10000
Ca	>150000
Cd	77
Cu	346
Fe	98800
K	1400
Li	8
Mg	2760
Mn	7727
Na	370
Ni	15
P	470
Pb	5772
S	>50000
Sb	443
Zn	>10000

ding heavy metals, high concentrations of lead (Pb: 5,772 ppm), zinc (Zn: >10,000 ppm), and arsenic (As: >10,000 ppm) were identified, indicating a potentially toxic environment for most microorganisms. However, such extreme conditions favor the presence of bacteria adapted to heavy metal resistance and metabolism, which is advantageous for bioremediation strategies and the recovery of valuable elements. Additionally, the elevated levels of iron (Fe: 9.88%) and sulfur (S: >5%) suggest favorable conditions for the proliferation of chemolithotrophic bacteria, which play a crucial role in the oxidation of metal compounds and the solubilization of strategic metals such as lithium [22, 23, 24].

3.2. Morphological characterization

The use of different selective media yielded different results. No bacterial growth was observed in 9K medium. In contrast, two distinct strains were isolated on LB medium: PBLB and PRLB. PBLB formed large-diameter colonies with a hard texture, grayish-white coloration, elevated structure, and a stellate shape, while microscopic analysis identified them as Gram-negative bacilli. PRLB exhibited medium-diameter colonies with a viscous texture, opaque pink coloration, a flat profile, and a wavy shape, also corresponding to Gram-negative bacilli. In API medium, two additional strains were obtained: AAPI and BAPI. AAPI formed small-diameter colonies with a viscous texture, yellow coloration, a circular shape, and a flat profile, being identified as Gram-positive bacilli under microscopic analysis. Finally, BAPI presented medium-diameter colonies with a viscous texture, opaque white coloration, a flat profile, and a stellate shape, also classified as Gram-positive bacilli based on microscopic characterization (Figure 2).

3.3. pH and redox characterization of the tailings

The redox potential (mV) was measured to assess the oxidative conditions of the experimental system. The negative values obtained indicate a highly oxidative environment, leading to the alkalinization of the medium—an expected outcome resulting from the interaction of metabolic byproducts generated by the bacteria involved (Figure 3). The relationship between redox potential and pH was influenced by continuous agitation, which played a crucial role in maintaining an optimal environment for aerobic bacteria by ensuring adequate oxygenation. This process not only promoted oxidative reactions but also directly influenced both parameters. The identified oxidative conditions support the hypothesis that the experimental setup favored the activity of aerobic microorganisms, particularly those involved in bioleaching and the oxidation of metal compounds. The observed alkalinization further validates the expected biochemical interactions and provides valuable insights into microbial adaptability to extreme environments (Figure 3) [25, 26].

3.4. Microbial growth kinetics and minimum inhibition

This study assessed the growth kinetics of five bacterial strains—AAPI, BAPI, PRLB, PBLB, and *E. coli* (used as a control)—and their response to different concentrations of lithium chloride (LiCl) in minimum inhibitory concentration (MIC) assays. LiCl was tested at three concentrations: 10,000 ppm, 15,000 ppm, and 20,000 ppm, revealing variability in strain tolerance.

In the control group, all bacterial strains exhibited growth without a distinct adaptation phase. PRLB showed the lowest growth, with optical density (OD) values ranging between 0.5 and 1, whereas the other strains reached higher values (Figure 4). At 10,000 ppm of LiCl, *E. coli* and PRLB did not exceed an OD of 1, while AAPI, BAPI, and PBLB maintained growth above 1.5 (Figure 4). At 15,000 ppm, *E. coli* and PRLB did not surpass an OD of 0.5, in contrast to the other strains, which continued growing above 1.5 (Figure 4). At 20,000 ppm, PRLB and *E. coli* exhibited complete growth inhibition (OD < 0.1), while BAPI displayed an adaptation period before resuming growth after 30 hours. AAPI and PBLB maintained stable growth, with OD values exceeding 1.5 (Figure 4).

These results indicate that PRLB and *E. coli* are highly sensitive to LiCl, whereas AAPI, BAPI, and PBLB exhibit greater resistance, maintaining sustained growth even un-

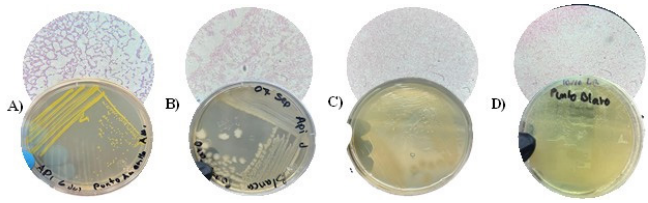

Fig. 2. Macroscopic and microscopic characterization of strains isolated from mining tailings AAPI, BAPI, PRLB, and PBLB. A) Gram staining and growth on solid media for AAPI. B) Gram staining and growth on solid media for BAPI. C) Gram staining and growth on solid media for PRLB. D) Gram staining and growth on solid media for PBLB.

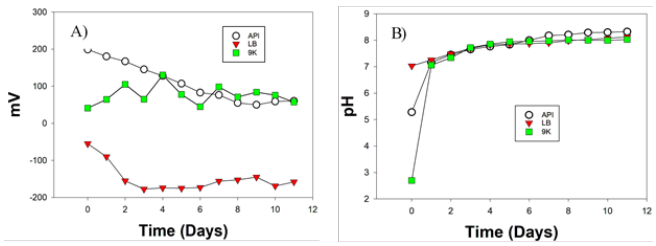

Fig. 3. A) Variation in mV values for the three media. B) Variation in pH values for the three media.

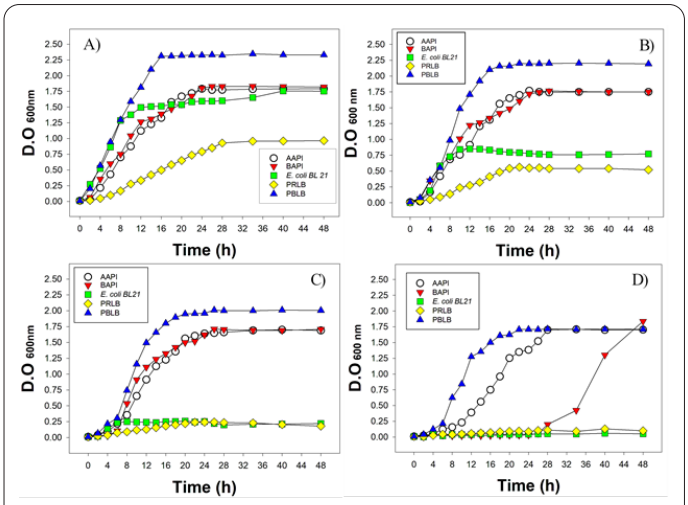

Fig. 4. Growth kinetics of the isolated strains, including *E. coli BL21*, in LB medium. A) Without LiCl supplementation. B) Supplemented with 10 g/L of LiCl. C) Supplemented with 15 g/L of LiCl. D) Supplemented with 20 g/L of LiCl.

der lithium-induced stress. These findings offer valuable insights into the adaptability and susceptibility of different bacterial strains to LiCl-rich environments.

3.5 DNA restriction with the enzyme *HhaI*

Figure 5 presents the 2% agarose gel electrophoresis results following DA purification. A 100 bp molecular marker was loaded in the first lane as a reference for estimating the size of the amplified DNA fragments in the subsequent lanes.

A DNA restriction analysis was performed using the *HhaI* enzyme to precisely identify bacteria with potential applications in bioleaching processes. The procedure began with a literature review focusing on reports of bacteria associated with resistance to heavy metals present in the analyzed mineral waste. Based on this information, candidate strains were selected, and their 16S rRNA gene sequences (≈1500 bp) were retrieved from the NCBI taxonomic database due to their critical role in bacterial identification. These sequences were then analyzed using the NEBcutter online software, which predicted the restriction sites targeted by the *HhaI* enzyme. The *in silico* digestion patterns obtained were compared with the *in vitro* results (Figure 5) generated from the analyzed samples [17, 27].

Figure 6 presents the comparison between the fragments obtained by enzymatic digestion with *HhaI* and the restriction patterns predicted by NEBcutter for different bacterial species. In the case of *Pseudomonas alcaligenes* (Figure 6.1), a high degree of concordance was observed between the experimentally obtained and predicted patterns, supporting its identification. This bacterium is notable for its ability to resist and transform heavy metals through bioaccumulation and biomineralization, which are key mechanisms in bioremediation and bioleaching [28, 29].

On the other hand, *Acinetobacter baumannii* (Figure 6.3) exhibited restriction fragments consistent with the predicted patterns, enabling its identification. This microorganism demonstrates resistance to metals such as cadmium, lead, and mercury, utilizing bioadsorption and the formation of non-toxic complexes—characteristics that make it valuable for environmental decontamination [30, 31].

In the analysis of *Bacillus cereus* (Figure 6.4), the obtained fragments aligned with those predicted *in silico*, facilitating its identification. This species tolerates metals such as cadmium and lead, immobilizing them through precipitation mechanisms and expressing resistance genes such as *copA* and *cadA*. These characteristics make it a promising candidate for the remediation of contaminated soil and water [32, 33, 34].

Similarly, in the enzymatic digestion of *Salmonella enterica* (Figure 6.5), patterns compatible with this species were identified. *S. enterica* exhibits resistance to metals such as copper, zinc, and chromium through the *czcD* and *pcoC* genes. These resistance mechanisms, associated with efflux systems, enable its adaptation to contaminated environments and highlight its potential application in bioremediation [35, 36].

The restriction analysis revealed that some cleavage patterns did not match those in bioinformatics databases, emphasizing the need for complementary studies. Additionally, identical patterns were observed in lanes 3 and 6 of Figure 6, suggesting that the strains analyzed may be genetically similar. These results demonstrate that enzymatic digestion serves as an effective presumptive tool for the initial characterization of bacteria with biotechnological potential in bioleaching and bioremediation. However, it is recommended to complement this method with DNA sequencing for definitive identification and more precise characterization.

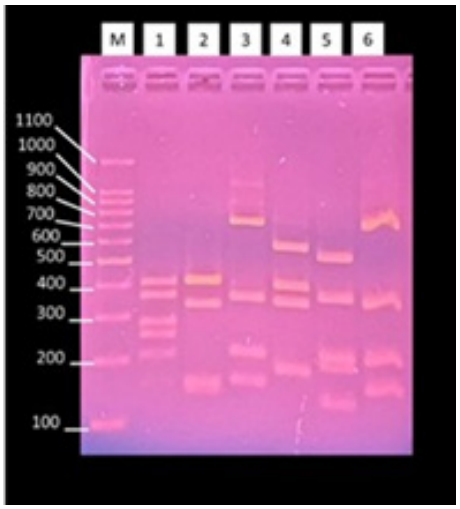

Fig. 5. Agarose gel electrophoresis of the enzymatic restriction of the isolated bacteria. M) Molecular marker 100 bp, 1) PRLB, 2) AAPI, 3) PBT, 4) BAPI, 5) PBO and 6) PBLB.

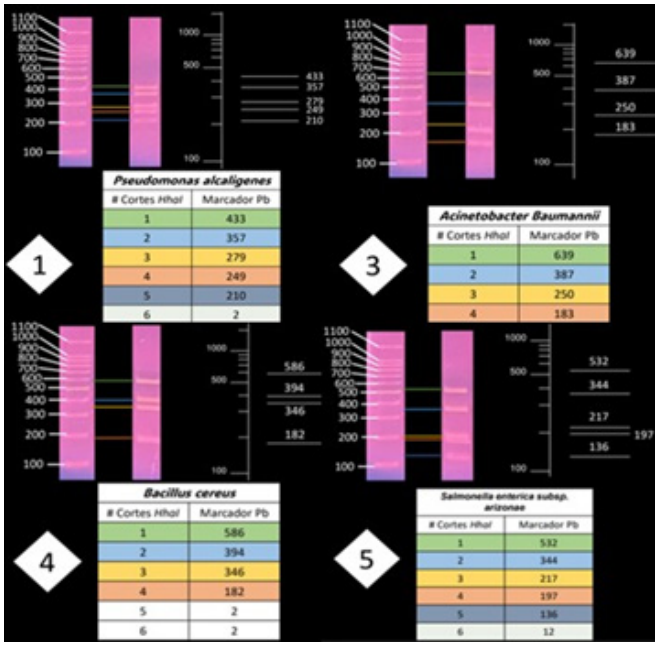

Fig. 6. Comparison of the fragments obtained by enzymatic digestion with *HhaI* on agarose gel (lane 1) and the predicted restriction patterns analyzed using NEBcutter: 1) PRLB, 3) PBT, 4) BAPI, 5) PBO.

3.6 Molecular characterization

The results obtained from the sequences of the five strains were aligned, and, based on this analysis, individual phylogenetic trees were constructed to determine the identity of each strain.

Figure 7A shows the phylogenetic tree of the KR24-PRLB strain, which has a similarity of 87%, and it was identified as *Stutzerimonas chloritidismutans*, a Gram-negative bacterium of the Pseudomonades genus, capable of

reducing chlorates and nitrates in anaerobic environments, adapted to surroundings with heavy metals and chlorinated compounds [37, 38]. Its potential in bioremediation is due to its genes associated with the detoxification and expulsion of heavy metals [39, 40].

The phylogeny of the KR24-LB strain in Figure 7B indicates a strong similarity (83%) to *Stenotrophomonas maltophilia*, an aerobic, antibiotic-resistant, Gram-negative bacterium belonging to the Stenotrophomonas genus. This bacterium tolerates heavy metals such as cadmium, zinc, and mercury, using processes such as reducing oxyanions to non-toxic forms, and is a candidate for bioremediation [41, 42].

Figure 7C shows the phylogenetic tree of the KR24-AAPI strain, identified as *Microbacterium paraoxydans* with a similarity of 80%. It is a Gram-positive bacterium, capable of removing heavy metals such as chromium, cadmium and lead with efficiencies greater than 80% [43]. Its adaptability to a wide range of temperatures and pH makes it suitable for natural and clinical environments.

Figure 7E presents the phylogenetic tree of the KR24-BAPI strain, which has a 79% similarity to *Bacillus thuringiensis*, a Gram-positive bacterium that produces δ-endotoxins, toxic to insects and is known for its resistance to heavy metals. Its use as a biopesticide and its ability to tolerate metals such as lead, chromium, and zinc position it as an option for environmental remediation [42, 43, 44].

Figure 7E shows the phylogenetic tree of strain KR24-PBO, which exhibits 81% similarity and was identified as *Enterobacter ludwigii*. This Gram-negative bacterium is resistant to metals such as cadmium, copper, and zinc, and has demonstrated biosorption and bioaccumulation mechanisms for detoxification [44, 46].

These strains, adapted to extreme concentrations of heavy metals, reinforce their potential for biotechnological applications in the bioremediation of contaminated soils and waters. The five bacterial strains isolated from the mining tailings of the Noche Buena mining company have been identified as species with a high capacity for resistance to heavy metals, which underlines their relevance in bioremediation applications in contaminated environments.

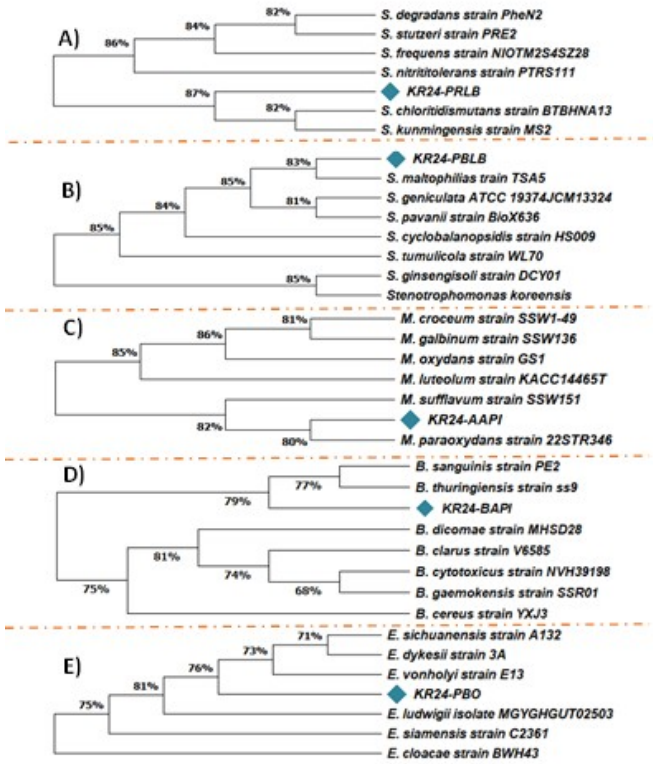

Fig. 7. Phylogenetic tree of the strains isolated from the mine tailings (Zacatecas), A) Strain KR24-PRLB, B) Strain KR24-LB, C) Strain KR24-AAPI, D) Strain KR24-BAPI, E) Strain KR24-PBO.

4. Discussion

In the present study, six native microbial strains were successfully isolated from lithium-contaminated mine tailings. These strains exhibited remarkable resistance to high concentrations of various heavy metals, including arsenic, iron, lead, manganese, copper, and zinc. These results suggest that the isolated microorganisms possess physiological and molecular adaptations that enable them to survive under extreme conditions, highlighting their potential for biotechnological applications, particularly in the bioremediation of contaminated environments.

Lithium resistance assays revealed that the isolated strains were capable of tolerating concentrations above 15,000 ppm of LiCl. This finding is significant as it confirms the ability of these bacteria to withstand highly toxic conditions, thereby opening the possibility of their use in lithium recovery processes from complex sources such as mine tailings. Furthermore, the observed resistance to other heavy metals suggests that these strains have developed broad-spectrum defense mechanisms, indicative of their ability to thrive in environments contaminated with multiple metals.

Molecular analysis through 16S rRNA gene sequencing enabled the identification of the isolates as *Stutzerimonas chloritidismutans, Stenotrophomonas maltophilia, Microbacterium paraoxydans, Bacillus thuringiensis,* and *Enterobacter ludwigii*. These results align with previous studies that have highlighted the capability of these microorganisms to resist and detoxify heavy metals such as lead, cadmium, and chromium. Additionally, the phylogenetic trees constructed during the bioinformatic analysis showed that the strains share genetic and physiological traits associated with metal resistance, reinforcing their potential use in biotechnological applications.

Given the tolerance to ~15,000 ppm LiCl observed in the analyzed strains, it is likely that they employ general metabolic mechanisms to survive and mobilize ionic metals. Certain species, such as *Stenotrophomonas, Microbacterium*, and *Bacillus*, may produce acidic compounds or exopolysaccharides (EPS) that facilitate the solubilization of lithium and other metals through processes such as acidolysis or redoxolysis—mechanisms similar to those observed in bioleaching bacteria like *Acidithiobacillus ferrooxidans* [47, 48]. These compounds can lower the pH of the surrounding microenvironment or act as chelating agents that capture metals, promoting their release from mineral matrices.

Notably, *Stenotrophomonas maltophilia* has been reported for its ability to form biofilms and produce biosurfactants that may aid in mineral dissolution [49]. *Microbacterium paraoxydans*, on the other hand, can tolerate hypersaline environments and participate in metal precipitation through specific metabolic pathways [50]. *Bacillus thuringiensis,* in addition to its well-known en-

tomopathogenic activity, has demonstrated the ability to biosorb metal ions and generate reducing conditions under anoxic environments [51]. Finally, *Enterobacter ludwigii* and *Stutzerimonas chloritidismutans* may facilitate redox reactions and metal immobilization through enzymatic pathways that warrant further investigation in the context of bioleaching [52].

The bioinformatic analyses, combined with comparisons to genetically related species, yielded valuable evolutionary insights, highlighting the remarkable adaptability of these strains to metal-contaminated environments. This adaptability is particularly significant, as it indicates that these microorganisms not only withstand the metals prevalent in their native habitats but also possess considerable potential for applications in bioremediation, metal recovery, and the mitigation of mining-related pollution. Collectively, the physiological, genetic, and functional profiles of the isolates emphasize their practical value in environmental biotechnology, opening new avenues for the sustainable exploitation of lithium-rich mining residues.

This study successfully isolated and characterized native microorganisms from lithium mining tailings, achieving its primary objectives. The isolates demonstrated exceptional resistance to lithium and other heavy metals, positioning them as promising candidates for biotechnological applications in bioremediation and metal recovery. Combined molecular and bioinformatic analyses further revealed their potential to reduce environmental contamination, underscoring their relevance for sustainable mining waste management. These findings not only expand our understanding of microbial adaptation to extreme environments but also pave the way for innovative, eco-friendly strategies in the valorization of lithium-rich residues.

Conflict of interests
The authors have no conflicts with any step of the article preparation.

Consent for publications
The authors read and approved the final manuscript for publication.

Ethics approval and consent to participate
No human or animals were used in the present research.

Informed consent
The authors declare that no patients were used in this study.

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Availability of data and material
The data that support the findings of this study are available from the corresponding author upon reasonable request

Authors' contributions
ZADE, RJD, and MRH designed the research study. ZADE, RJD, MRH, VOPG, RCJA, MRGA, and ROKS performed the research. ZADE, RJD, VOGP, RCJA and ROKS analyzed the data. ZADE, RJD, VOPG, RCJA, MRGA, and ROKS wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors have read and approved the final manuscript

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