

Chemical Characterization of Mine Water and Microbial Communities from the Mária Mine (Rožňava, Slovakia): Assessment of Autotrophic and Heterotrophic Bacteria after Cultivation on Liquid and Solid Media

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Abstract

The Mária Mine (Rožňava) represents an important deposit of Ag-bearing tetrahedrite rich in Cu and Sb. The mine drainage discharges into the Slaná River, underscoring the need to characterize its chemical composition and bacterial community. Mine water chemistry was evaluated by ICP spectrometry and ion chromatography. Microorganisms from the Mária mine drainage were cultivated in liquid 9K medium and on solid gelrite medium and subsequently identified using whole-genome sequencing. The mine drainage was characterized by neutral pH (6.9) and elevated sulfate, Mn and Fe concentrations. Cultivation in liquid medium revealed the iron-oxidizing bacterium *Acidithiobacillus ferrooxidans*. Solid gelrite medium yielded heterotrophic bacteria affiliated with *Pseudomonas*.

Keywords: Mária mine, Tetrahedrite, bacterial identification, *Acidithiobacillus*, *Pseudomonas*

Introduction

The Mária Mine in Rožňava, located along the Strieborná vein, represents one of Slovakia's most important deposits, characterized by Ag-bearing tetrahedrite with approximately 40–46 wt% Cu, 26 wt% Sb, and ~1 wt% Ag. This mineralization makes the site a valuable source of strategic and critical raw materials. Studying this mine drainage is particularly important, as it discharges into the Slaná River, which flows beyond the Slovak border into Hungary, underscoring the need to characterize its chemical composition and microbial community to better understand potential environmental impacts and evaluate its biotechnological relevance (Kupka *et al.* 2025; Hagarová & Kupka 2025).

Tetrahedrite represents a copper antimony sulfosalt that can be described by the simplified chemical formula $\text{Cu}_{10}(\text{Fe,Zn})_2\text{Sb}_4\text{S}_{13}$. In natural systems, the minerals of the tetrahedrite group form a complex isotopic series with multiple iso- and heterovalent substitutions (King 2001; Moëlo *et al.* 2008). Tetrahedrite-group members

may contain variable amounts of arsenic, antimony, mercury, or cadmium. Therefore, the waste from these deposits may pose environmental challenges.

Oxidative exposure of tetrahedrite and tennantite can lead to the mobilization of Sb, As, Cu, S, and other structurally bound elements. As a potentially toxic metalloid, Sb is released into the environment through multiple pathways, including mining activities, ore transport and processing, smelting operations, production and use of products, waste and sludge disposal, and industrial effluents (Liu *et al.* 2023; Stančič *et al.* 2022).

In this context, bioleaching tests were conducted to assess the behavior of the tetrahedrite concentrate under controlled acidic, oxidizing conditions at 25°C. Tetrahedrite bioleaching by acidophilic iron- and sulfur-oxidizing bacteria led to recovery of >80% Cu (>4 g L⁻¹). In addition, Sb was immobilized via precipitation of Sb(V) phases (Kupka *et al.* 2025). Although this mineral has typically been processed by alkaline



sulfide leaching due to its refractory behavior in acidic ferric media (Awe *et al.* 2010; Baláž & Achimovičová 2006), the present results demonstrate that bioleaching at ambient temperature can achieve efficient copper recovery without alkaline pre-treatment (Kupka *et al.* 2025).

To complement these process-based findings, the first comprehensive shotgun metagenomic survey of this mine drainage was conducted. The analysis generated approximately 227 million high-quality reads and revealed a community dominated by Betaproteobacteria (> 66%), with abundant lithotrophic genera *Sulfuritalea* (6.93%), *Ferrigenium* (5.45%), *Gallionella* (3.79%), and *Sideroxydans* (3.65%), alongside the heterotrophic genus *Pseudomonas* (5.2%). Among the most prevalent neutrophilic iron-oxidizing bacterial strains were *Sulfuritalea hydrogenivorans* (6.93%), *Ferrigenium kumadai* (5.45%) and *Gallionella capsiferriformans* (3.79%). Acidophilic genera (*Thiobacillus* sp. at 0.43%, *Ferrovum myxofaciens*, *Acidithiobacillus ferrivorans*, *Leptospirillum ferrooxidans*) collectively accounted for <1% of the community (Hagarová & Kupka 2025).

A key tool for mapping genomes of novel microorganisms is represented by whole-genome sequencing (WGS) that offers the ability to interrogate the entire DNA sequence of the genome without the need to use selective capture techniques to target and amplify a specific gene (Haworth *et al.* 2016). Sequencing the entire microbial genome is crucial for precise microbial identification and generation of complete reference genomes. Metagenomic data provide reliable estimates of bacterial community composition and diversity without the need to target and amplify a specific gene (Pérez-Cobas *et al.* 2020).

In this study, bacterial identification was performed following the cultivation of microorganisms originating from the Mária mine drainage in Rožňava on both liquid and solid media.

Methods

The mine water sample was collected from the outdoor discharge pipe of the Mária mine

(Rožňava) (Fig. 1). Mine water chemistry was analyzed *in situ* using a pH meter (Denver Instrument UltraBasic) and a conductometer (WTW Cond 330i Set, TetraCon 325). Ion and metal concentrations were determined by ICP spectrometry and ion chromatography (Dionex ICS 5000, Sunnyvale, CA, USA).

The mine water, collected under sterile conditions, was used as an inoculum in liquid 9K medium containing 33.36 g L⁻¹ FeSO₄ · H₂O (Silverman & Lundgren 1959) and on solid gelrite medium of similar composition but with reduced Fe²⁺ concentration (0.02 g L⁻¹) to capture heterotrophs. DNA from cultivated isolates was extracted using the QIAamp BiOstic Bacteremia DNA Kit (Qiagen, Germany).

Genomic DNA was subjected to whole-genome sequencing using an approach combining short-read sequencing (DNBSEQ platform, BGI Tech, China) and long-read sequencing (Oxford Nanopore platform). Raw reads were filtered to remove low-quality sequences, adapters, duplicated reads, and short fragments prior to assembly (Canu v2.2). For taxonomic identification and species verification, the assembled genome was aligned against the NCBI Nt (Nucleotide) database using BLAST-based similarity analysis. GC content and GC-depth correlation analysis were also performed to assess genome homogeneity and exclude potential contamination.

Results

Chemical characterization of mine water

Abiotic data revealed that the Mária mine releases a distinct neutral-pH (6.9) drainage enriched in metals, containing approximately 403.12 mg L⁻¹ SO₄²⁻, 5.58 mg L⁻¹ Mn, and 4.66 mg L⁻¹ Fe (Tab. 1). The temperature of the water sample was 13.9 °C, with a conductivity of 1,029 µS cm⁻¹, respectively.

Bacterial cultivation in liquid 9K medium

Cultivation in liquid 9K medium resulted in enrichment of an iron-oxidizing bacterium identified as *Acidithiobacillus ferrooxidans* (cover length 1,753,528 bp; coverage 99.09%; genomics 70%). Additional lower-percentage matches to related taxa were observed but covered substantially smaller

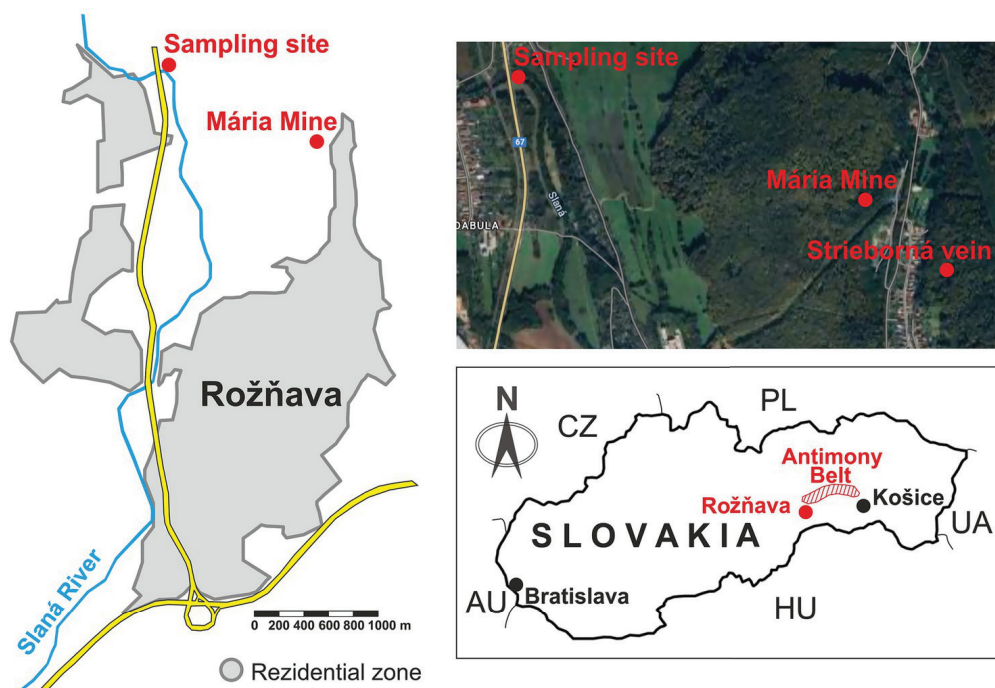


Figure 1 Sampling site and the Mária mine in Rožňava (Hagarová & Kupka 2025).

Table 1 Chemical composition of the Mária mine water drainage.

Parameter	SO ₄ ²⁻	Mg ²⁺	Ca ²⁺	Cl ⁻	Na ⁺	K ⁺	Mn	Fe	F ⁻
mg L ⁻¹	403.12	80.88	80.23	34.49	31.9	5.71	5.58	4.66	0.41
Parameter	NH ⁴⁺	Li ⁺	As	Ni	Co	Al	Zn	Sb	Cu
mg L ⁻¹	0.242	0.032	0.01	0.01	0.006	0.006	0.006	0.005	0.004

genomic fractions, supporting the primary classification.

The final assembled genome had a total size of 1,850,565 bp with an overall GC content of 57.77%. The genome comprised one chromosome (1,644,415 bp; 58.04%) and five plasmids. Sequencing depth reached approximately 688x based on short-read data (1,275 Mb) and 1621x based on long-read data (3,000 Mb), providing strong support for the completeness and reliability of the genome reconstruction. The Nanopore long-read dataset was characterized by read lengths predominantly between 1-10 kb, with a maximum read length of 75,515 bp (Fig. 2).

Bacterial cultivation on solid gelrite medium

Taxonomic analysis based on Nt database comparison revealed the presence of multiple species affiliated with the genus *Pseudomonas*. Solid gelrite medium yielded heterotrophic bacteria predominantly assigned to *Pseudomonas* (*P.*) *fluorescens* (cover length 12,091,501 bp; coverage 82.66%; genomics 47.22%), together with genomic fractions closely related to *P. azotoformans* or *P. putida*, indicating the presence of more than one *Pseudomonas* species within the bacterial sample.

Genome assembly of the bacterial sample resulted in 5,258 contigs with a

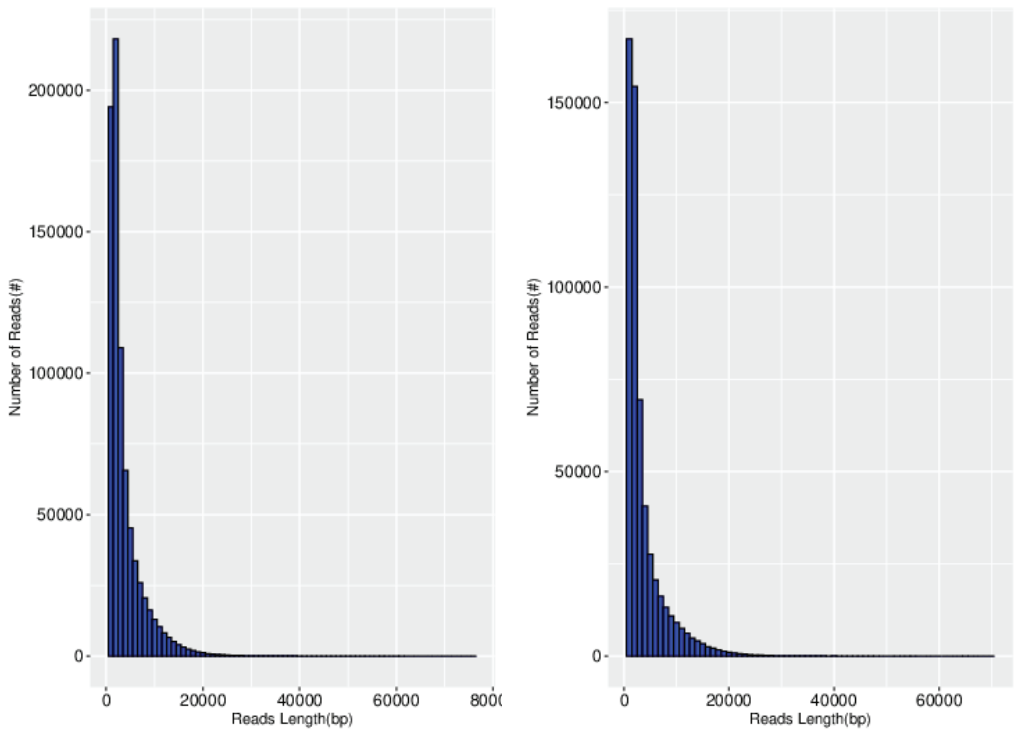


Figure 2 Clean reads length distribution, Left – bacterial sample cultivated in liquid 9K medium; Right – bacterial sample cultivated on solid gelrite medium.

total genome size of 30,976,816 bp and an overall GC content of 59.63%. The nanopore dataset comprised 567,178 reads, with a maximum read length of 70,102 bp (Fig. 2). The combined sequencing depth reached approximately 40x for short reads and 67x for long reads.

Conclusions

Abiotic characterization confirms that the Mária mine drainage represents a metal-enriched, neutral-pH environment that provides the geochemical framework for the development of distinct microbial functional groups.

The contrasting genomic profiles obtained from two cultivation approaches reflect the selective pressure imposed by growth conditions. Enrichment in liquid 9K medium, designed to favor iron-oxidizing chemolithotrophs, resulted in a near-complete and highly homogeneous genome of *A. ferrooxidans*, supported by high sequencing depth and a low number

of replicons. In contrast, cultivation on a solid gelrite medium favored heterotrophic growth and resulted in a more genetically diverse bacterial population affiliated with multiple *Pseudomonas* species. These findings demonstrate that cultivation conditions strongly influence the composition and genomic architecture of the recovered microbial fraction.

Understanding these cultivation-driven shifts is essential for correctly interpreting microbial community structure and functional potential in mine-affected environments. In such systems, both iron-oxidizing autotrophs and metabolically versatile heterotrophs contribute to key biogeochemical processes.

The functional attributes may also indicate potential application pathways, although they are inferred from metagenomic data and require experimental validation. Under the circumneutral pH conditions of the Mária mine drainage, the dominant neutrophilic



iron-oxidizing bacteria are likely to play a more important role in iron cycling than acidophilic taxa such as *Acidithiobacillus*, whose low abundance and cultivation-based enrichment may overestimate their environmental relevance. Heterotrophic taxa such as *Pseudomonas* are likely to contribute primarily through the degradation of organic matter or siderophore-mediated iron mobilization. The presence of metal-resistance determinants (e.g., Czc, Ars, Aio) may also be relevant for metal tolerance and detoxification at moderate metal concentrations (Hagarová & Kupka 2025).

This work is essential for establishing the site's geochemical context and evaluating its microbial potential, particularly the presence of iron-oxidizing bacteria relevant to biohydrometallurgical processes. Such microorganisms enable the solubilization of metals from sulfide minerals (e.g., tetrahedrite) under environmentally sustainable conditions. The findings underscore the dual importance of monitoring mine water for environmental protection and exploring its microbial resources for innovative, low-impact extraction technologies.

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