

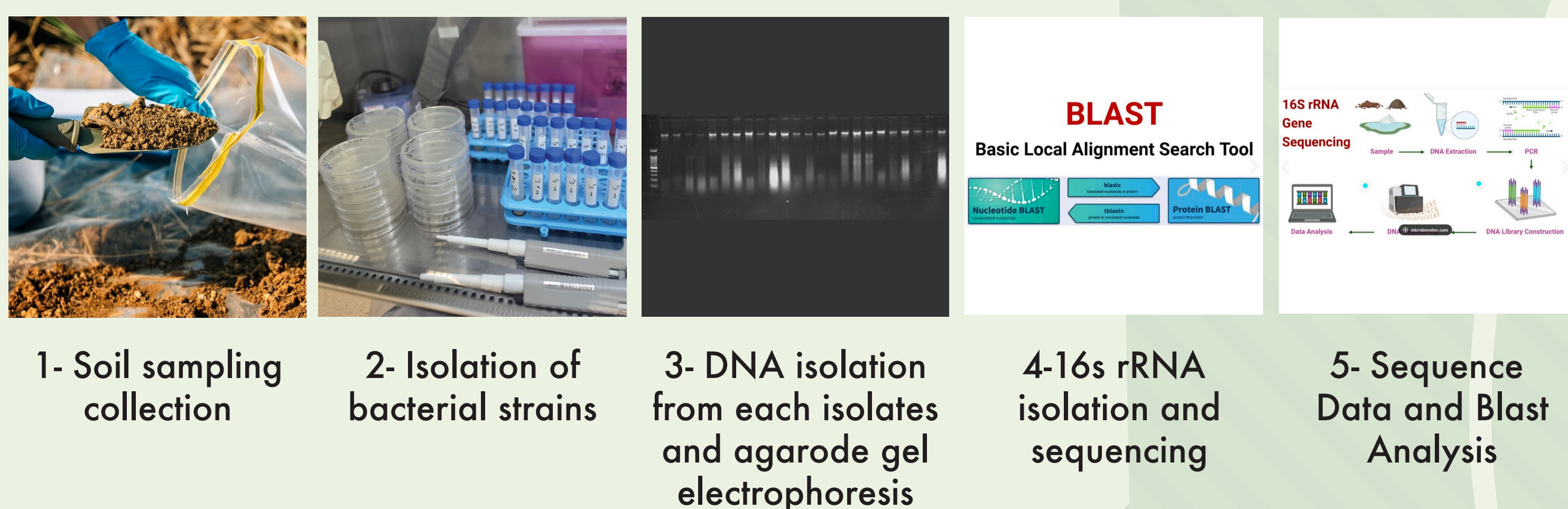
# Isolation and Molecular Identification of bacterial strains isolated from gold mines

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## Background

Soil is one of the most diverse microbial habitats on Earth, harboring complex communities of bacteria, fungi, and archaea that are central to nutrient cycling, and ecosystem stability (Bach, et al., 2018; Fierer, 2017). Understanding the taxonomic composition and genetic diversity of soil bacteria is therefore essential for unraveling their ecological roles and for exploring potential applications in biotechnology especially in mining research. Molecular tools, particularly the sequencing of the 16S rRNA gene, provide a robust framework for the identification and phylogenetic placement of bacterial isolates, enabling detection of both dominant and rare taxa in soil environments ( Xu et al., 2025; Janda & Abbott, 2007). In this study, partial 16S rRNA sequences were obtained from mine soil bacterial isolates and analyzed through a Bioinformatics tools through BLASTn searches, comparative analyses of sequence features such as length and GC content for genomic characterization and Phylogenetic trees were reconstructed using Neighbor-Joining methods with bootstrap support to assess evolutionary relationships. This study were done to identify isolated bacterial strains and to select the best suitable strain will be used in genetic improvement for gold bioleaching.

## Materials and Methods



1- Soil sampling collection

2- Isolation of bacterial strains

3- DNA isolation from each isolates and agarose gel electrophoresis

4-16s rRNA isolation and sequencing

5- Sequence Data and Blast Analysis

## Discussion

In Saudi Arabia and in other parts of the world, gold metal is considered one of the most important sources of the economy (Samontaray and Alanuzi, 2015). Gold ore is the most widely used metal aimed at a variety of consumer products. Bacterial communities constitute the oldest members of living adaptability systems in mining soils and thrive in adverse conditions and extreme ecosystems (Navas, et al., 2020; Fernandes et al., 2018). DNA sequence analysis introduces an efficient tool for understanding the evolutionary forces that shaped nucleotide variations as well as bring insight into the significance of specific genomic regions (Alkhowaiter et al., 2023).

In this study we noted that almost all high bacteria diversity and its GC varies within and between same species, it give us largely thought about a number of variables in connection with evolutionary history as well as the environment . This diversity within species variation is due to mutations (Brown 2002). Gene flow from one population to another produce additions and rearrangements of genomic regions (Thomas et al., 2005). Transferred DNA between cells can be done by horizontal gene transfer via transformation, transduction, conjugation (Lawrence et al., 2009). Phylogenetic analysis highlights both the stability of closely related strains and the broader taxonomic diversity present in soil bacterial communities as discussed by (Venail and Vives 2013).A clustered heatmap of the pairwise genetic distances among isolated bacterial species representing the highest and lowest genetic similarity between the genomes. This study is observed in the (Fernandes et al., 2018).

## Results

Isolated strains:

56 bacterial isolates were isolated from soil samples as illustrated in phylogenic tree Fig(4).

Successful DNA isolation from each bacterial strain were done as shown in Fig(2).

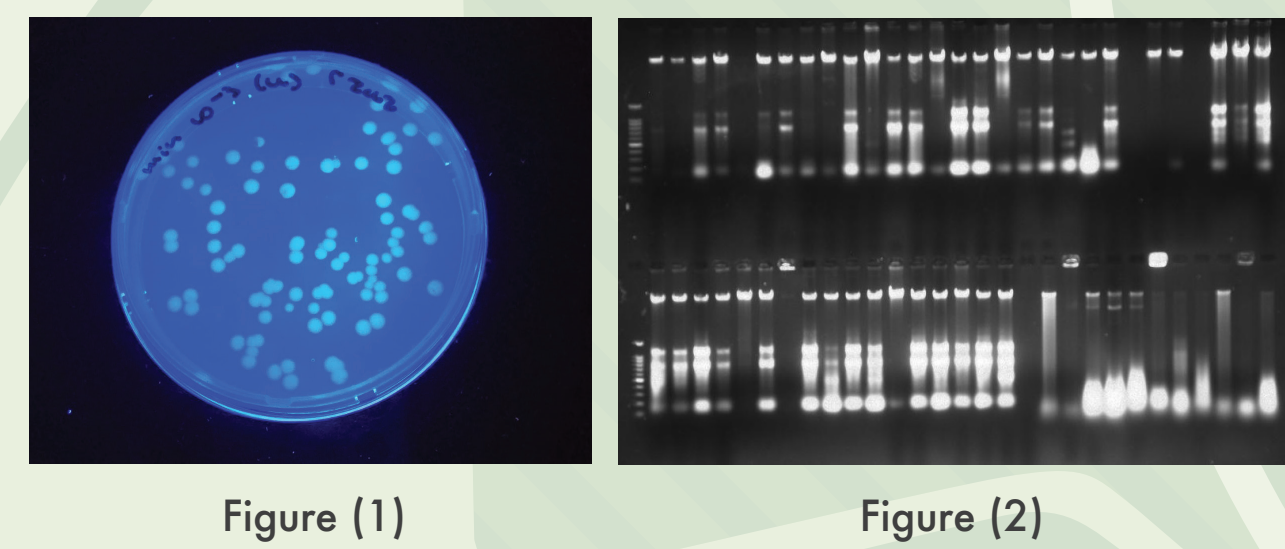


Figure (1)

Figure (2)

The 16S rRNA gene sequences from soil bacterial isolates were analyzed using BLAST against the NCBI database, and species-level identification was considered reliable for matches with  $\geq 98\%$  identity and  $\geq 95\%$  coverage. Most isolates showed high similarity (up to 100% identity), confirming that this approach provides strong taxonomic resolution, though closely related species may still be ambiguous. This dataset offers a baseline for studying genomic diversity and evolutionary relationships in soil bacteria.

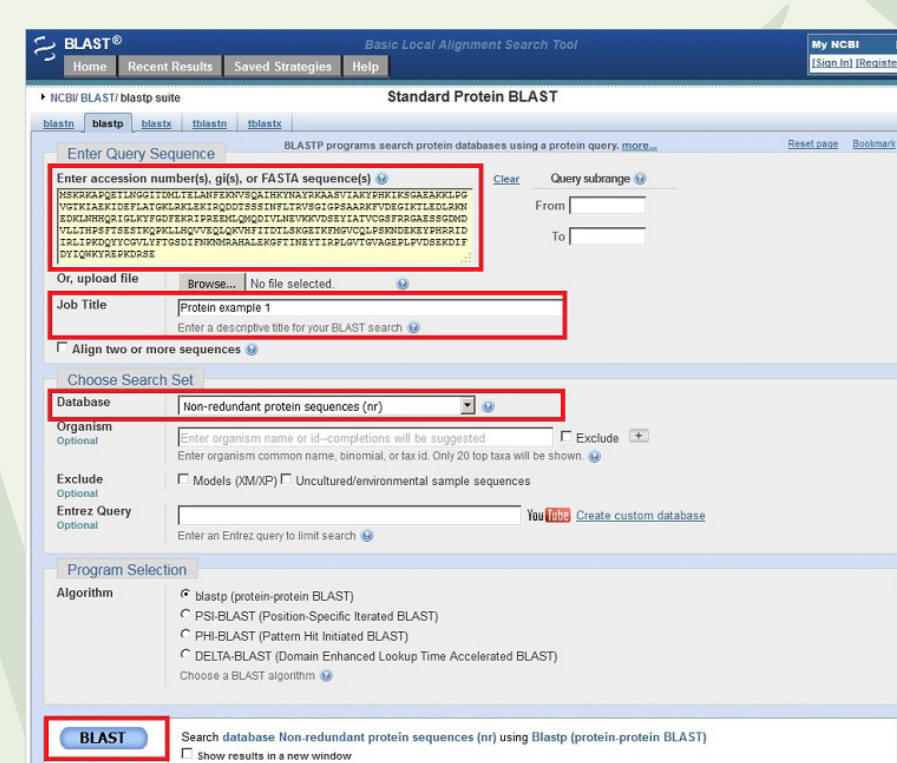


Figure (3)

Fig(4) shows a Neighbor-Joining phylogenetic tree of 56 soil bacterial isolates, clustering them by genus and illustrating genetic relationships. Branch lengths indicate divergence, with groups like Streptomyces showing higher variation, highlighting both the stability of closely related strains and the broader diversity of soil bacterial communities.

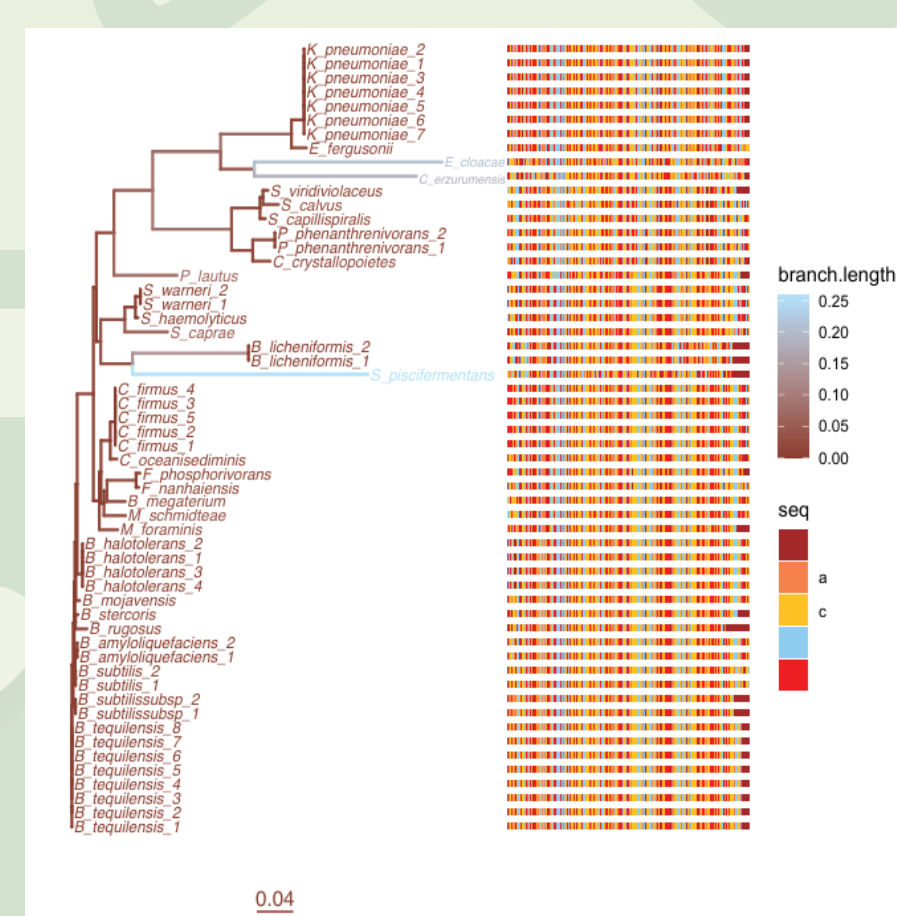


Figure (4)

Fig(5) presents the base composition (A, C, G, T) of 56 soil bacterial isolates, showing that nucleotide distributions are highly uniform within species while subtle GC content differences exist between species. These patterns align with genomic statistics like contig length and emphasize both the stability of closely related strains and the broader taxonomic diversity, providing a baseline for functional and evolutionary studies.

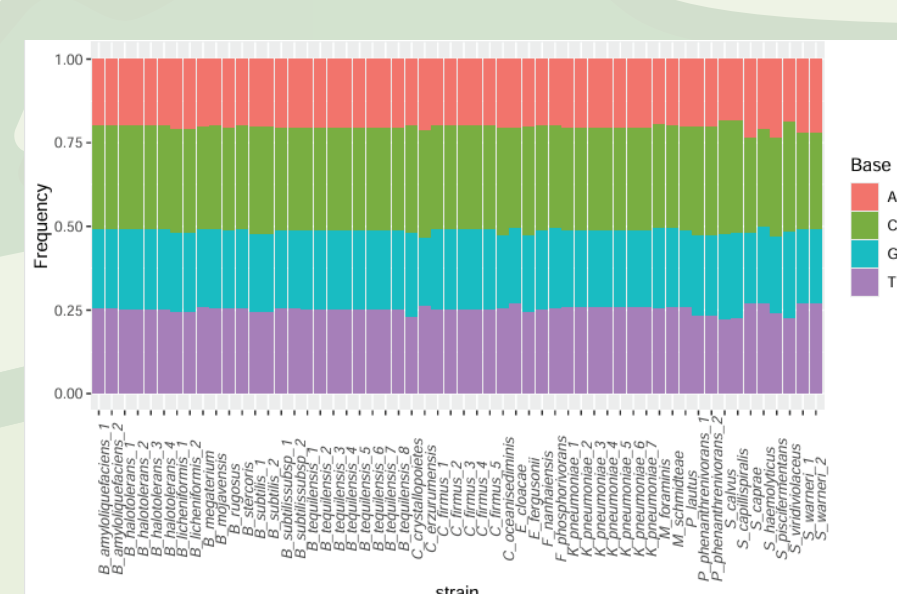


Figure (5)

Fig(6) shows a clustered heatmap of pairwise genetic distances among 30 haplotypes (H1-H30), with dendrograms grouping closely related haplotypes and highlighting more divergent ones. Darker colors indicate low divergence, while lighter shades show higher distances, providing a clear overview of haplotype relationships that complements the Neighbor-Joining phylogenetic tree.

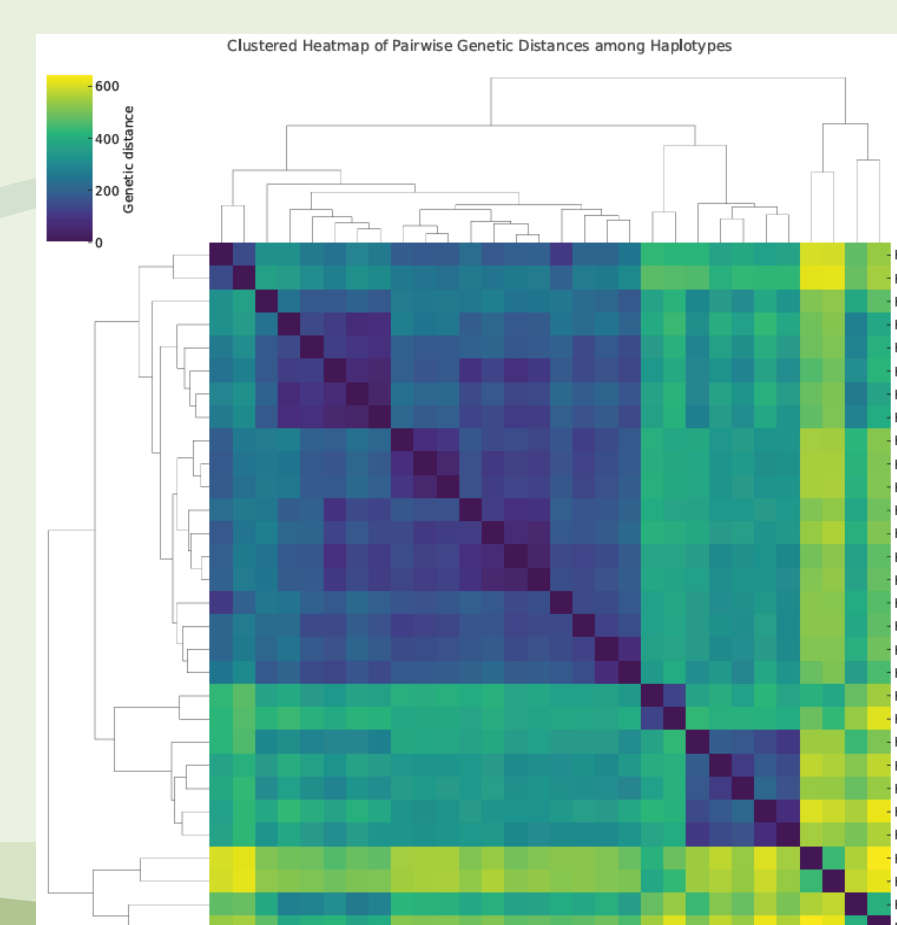
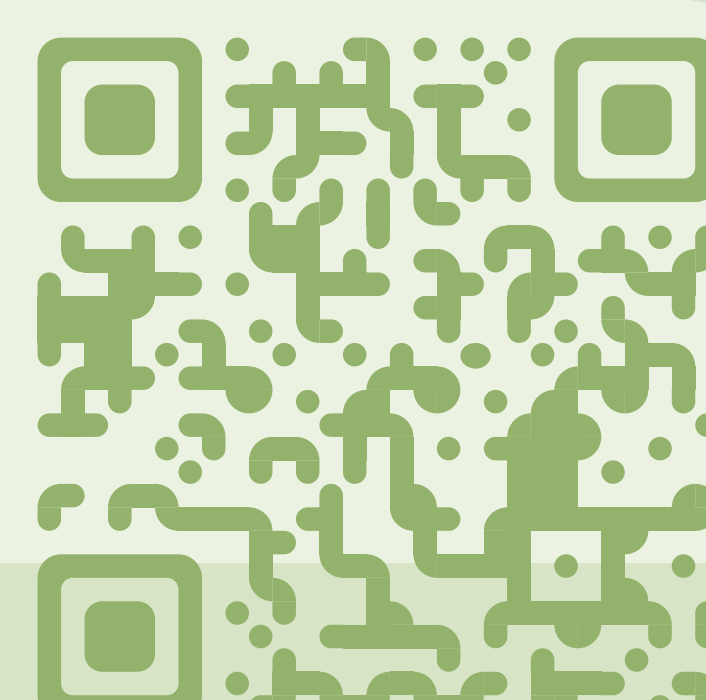


Figure (6)

## Conclusion

This study provides a comprehensive characterization of soil bacterial isolates using an integrative approach that combines sequence-based identification, phylogenetic reconstruction, haplotype analysis, and clustering visualization. Taxonomic assignments based on partial 16S rRNA gene sequences and BLAST searches enabled the reliable identification of several bacterial genera. Comparative genomic features, such as sequence length and GC content, further supported species-level differentiation. Phylogenetic analyses using Neighbor-Joining and bootstrap support values revealed robust clusters of closely related species alongside divergent lineages, providing insight into both recent diversification events and deeper evolutionary separations. Overall, the findings demonstrate that soil bacterial communities exhibit both stability within dominant taxa and heterogeneity across populations, reflecting their ecological complexity. This work provides a framework for studying microbial diversity in complex environments and contributes to a better understanding of the evolutionary and ecological dynamics shaping of mine soil bacterial strains. Future studies linking genetic diversity with functional traits will be essential to fully unravel the ecological significance of these bacterial lineages. This study also, gave us an idea of the types of bacteria that live in gold mines and overview of the best isolated strain will be select in next work in genetic improvement for gold bioleaching.

## For contacts



## Reference

