



Hidden Microcosmos in Slovak Gold Mine Rozalia – Microbial Gold Miners?

Lenka MALINIČOVÁ¹⁾, Lea NOSÁLOVÁ²⁾, Ivana TIMKOVÁ³⁾, Peter PRISTAŠ⁴⁾, Jana SEDLÁKOVÁ-KADUKOVÁ⁵⁾

¹⁾ Department of Microbiology, Institute of Biology and Ecology, Faculty of Science, Pavol Jozef Šafárik University in Košice, Šrobárova 2, 04154 Košice, Slovakia; email: lenka.malinicova@upjs.sk

²⁾ Department of Microbiology, Institute of Biology and Ecology, Faculty of Science, Pavol Jozef Šafárik University in Košice, Šrobárova 2, 04154 Košice, Slovakia; email: nosalova.lea11@gmail.com

³⁾ Department of Microbiology, Institute of Biology and Ecology, Faculty of Science, Pavol Jozef Šafárik University in Košice, Šrobárova 2, 04154 Košice, Slovakia; email: ivana.timkova@student.upjs.sk

⁴⁾ Department of Microbiology, Institute of Biology and Ecology, Faculty of Science, Pavol Jozef Šafárik University in Košice, Šrobárova 2, 04154 Košice, Slovakia; email: jana.sedlakova@upjs.sk

⁵⁾ Department of Microbiology, Institute of Biology and Ecology, Faculty of Science, Pavol Jozef Šafárik University in Košice, Šrobárova 2, 04154 Košice, Slovakia; email: peter.pristas@upjs.sk

<http://doi.org/10.29227/IM-2020-01-47>

Submission date: 02-01-2020 | Review date: 01-04-2020

Abstract

Biogeochemical cycling of gold involves dispersion and reconcentration of gold (Au) due to physical, chemical and biological processes in Earth surface environments. These processes are evoked by a metabolic activity of different microbial taxa but many of them (and also their biogeochemical potential) are still unexplored. Understanding the gold cycling is necessary for developing innovative, environmentally friendly gold processing techniques. Our experiments were aimed on isolation and identification of heterotrophic bacteria from ore and ore storage dump samples collected in Rozalia gold mine in Hodruša-Hámre. Using culture-based approach followed by combination of MALDI-TOF MS protein profiling and 16S rDNA sequencing, 18 different bacterial genera were identified in studied microbiota. The participation of several representatives of these genera in individual gold cycling steps has already been reported. The real involvement of bacterial isolates in gold transformation reactions and their biogeochemical potential will be studied in subsequent experiments.

Keywords: gold mine, biogeochemical cycle, bacterial diversity

Introduction

Geomicrobiology is the scientific field studying the role that microorganisms have played in the geologic past from the time of their first appearance on our planet to the present, the role they are playing today and will probably play in the future in some of the geologically important processes (Ehrlich and Newmann, 2009). Microorganisms participate in many processes including biotransformation of metals and minerals, as well as related substances, and they are intimately involved in metal biogeochemistry with a variety of mechanisms determining mobility and bioavailability (Gadd, 2010).

Gold (Au) is one of the rarest metals on earth. Based on its increased global demand in industry and nanotechnology, the need to supply Au will continue well into the future, despite the increasingly reduced availability of conventional economically and environmentally-viable sources. Therefore, searching for new gold deposits in the nature has become very important. On the other hand, waste products from several industrial processes contain residual gold, that could be recovered and reused. There is an essential need to develop alternative cost-effective and environmentally friendly methods for recovering gold from waste products (Lengke et al., 2006). Research in gold geomicrobiology has developed extensively over the last decade, more and more mechanisms are being discovered by which microbes interact with gold

in its biogeochemical cycle. In weathering environments, Au is mobile, taking the form of oxidized, soluble complexes or reduced, elemental Au nanoparticles. There is still growing evidence that microbes can directly influence solubilization, Au-nanoparticle formation, nanoparticle aggregation, and Au (re)distribution within the natural environment (Shuster and Reith, 2018). These microbial abilities make them suitable tool for biomining - inexpensive and ecological way to extract gold (and other precious metals) from metal-containing ores, waste products and concentrates using microbiological technology.

Ore mining and capacities of ore deposits in Slovakia, formerly very important, are in quite difficult situation nowadays. During last years the gold mining in Slovakia has been complicated due to objective economic conditions resulting from changes at the world metal exchange. Especially, it was the extensive variation of the price and sudden decline of the demands to produce gold that caused the difficulties with the gold mining in Hodruša-Hámre (Bauer et al., 2002).

The Rozalia mine (48°27'N, 18°51'E) at Banská Hodruša, located in the Middle Miocene Štiavnica stratovolcano on the inner side of the Carpathian arc in Slovakia, is the last operating ore mine in Slovakia. Since 1992 the base- and precious metal mineralization has been mined by the company Slovenská banská, Ltd., with variable annual production from 70 to 500 kg of gold. The eastern part of the deposit currently

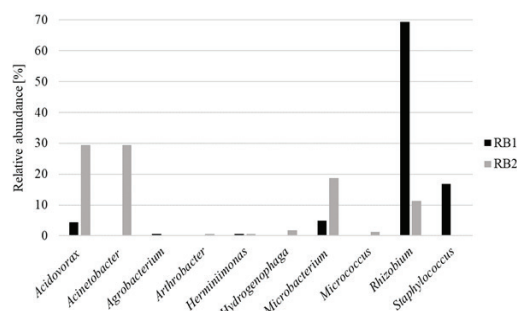


Fig. 1. Genus-level distribution of bacterial isolates obtained from two underground vein exposures RB1 and RB2
Rys. 1. Rozkład rodzaju izolatów bakteryjnych uzyskanych z dwóch podziemnych żył RB1 i RB2

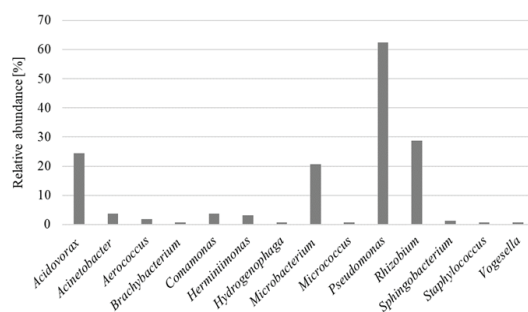


Fig. 2. Genus-level distribution of bacterial isolates obtained from ore storage dump sample H1
Rys. 2. Rozkład rodzaju izolatów bakteryjnych uzyskanych z próbki H1 składowiska rudy

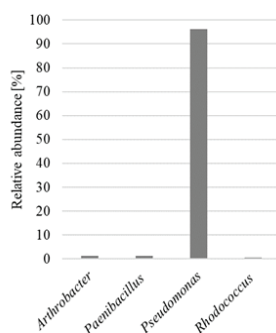


Fig. 3. Genus-level distribution of bacterial isolates obtained from soil sample P1
Rys. 3. Rozkład rodzaju izolatów bakteryjnych uzyskanych z próbki gleby P1

has an annual production of approximately 30–45 kt of ore containing 450–500 kg of Au (Kubač et al., 2018). The residual material from the ore treatment stored in the ore storage dump at Hodruša-Hámre still contains some residual gold, which is not efficient to recover by conventional processes. The aim of this work was to isolate and identify autochthonous heterotrophic bacteria from both subsurface mine environment and above-ground sites associated with the mine.

Materials and methods

Samples of ore material from two underground vein exposures in the XIV level of the Rozália mine (sample RB1 – two days after exposure and RB2 – one month after exposure), one sample (H1) from ore storage dump and one sample of soil (P1) from the site immediately near mine entrance were collected under sterile conditions.

1.0 g of each sample was mixed separately with 10 mL of sterile phosphate buffered saline, decimal dilution of samples was prepared and 100 μ L aliquots of all dilutions were spread

in parallels on four types of culture media (TSA – Trypticase Soy Agar, NA2 – Nutrient Agar no. 2, 10 \times NA2 – 10 \times diluted Nutrient Agar no. 2, and R2A – Reasoner's 2A agar). Cultivation was performed overnight at room temperature under aerobic conditions. From each of studied sample collection sites and each type of culture media, 40 randomly selected bacterial colonies were isolated and subcultured (640 colonies in total).

All bacterial isolates were subjected to identification using MALDI-TOF mass spectrometry protein profiling according to the manufacturer's manual (Microflex LT, Bruker Daltonics, Germany). Total bacterial DNA from isolates unidentified by MALDI-TOF MS was subsequently extracted using GenEluteTM Bacterial Genomic DNA kit (SIGMA-ALDRICH, Germany). Obtained DNA samples were used as templates for PCR amplification of 16S rRNA gene. PCR amplification was performed on C1000TM Thermal Cycler (BIO-RAD Laboratories, USA) and for preparation of reaction mixtures Taq Core kit/high yield (JENA Bioscience,

Tab. 1. Bacterial genera with proven participation in the biogeochemical cycle of gold
 Tab 1. Rodzaje bakterii o udowodnionym udziale w biogeochemicznym cyklu złota

Bacterial genus	Potential effect on Au-cycling process		Reference
<i>Arthrobacter</i> <i>Microbacterium</i> <i>Micrococcus</i> <i>Staphylococcus</i>	Mediation of initial colonization of the Au-grains surface		Rea et al., 2016
<i>Acinetobacter</i> <i>Pseudomonas</i>	Biofilm growth and recruitment	Stabilisation of the biofilm through the production of extracellular polymeric substances	Pal and Paul, 2008
<i>Rhizobium</i> <i>Acidovorax</i> <i>Pseudomonas</i>		Nitrogen fixation N ₂ cycling and continuous supply of usable nitrogen to other biofilm organisms	Rea et al., 2016
<i>Acinetobacter</i> <i>Arthrobacter</i> <i>Pseudomonas</i>		Metabolic turnover of complex organics, xenobiotic and toxins	
<i>Pseudomonas</i>	Au solubilization Precipitation of gold colloids		Reith et al., 2006

Germany) was used. Each PCR reaction mixture (50 µL) contained 1 µM of fD1 primer, 1µM of rP2 primer (Weisburg et al., 1991) and 50 ng of DNA template. The PCR included an initial denaturation at 94°C for 5 minutes followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 53°C for 1 minute, and extension at 72°C for 1 minute 30 seconds and final extension at 72°C for 10 minutes. Amplified fragments were purified using Wizard SV Gel and PCR Clean-Up System (Promega, USA). Sequencing of PCR amplicons was performed using both primers at Eurofins Genomics, Germany. The obtained sequences were assembled and the entire 16S rRNA gene sequences were subjected to BLAST search against GenBank database.

Results and discussion

From the biological point of view mines represent an extreme environment with low content of nutrients and often high concentration of metals. Our results showed surprisingly presence of many different bacterial genera inhabiting both subsurface and above-ground sites associated with the mine.

We expected that the variability of microorganisms would be higher in the above-ground environments (H1 and P1), which are influenced by external factors (weather conditions, contact with macro-organisms, etc.). In line with our expectations the highest genus-level distribution (14 genera) was observed in the ore storage dump sample H1 (Fig. 2), 6 and 8 bacterial genera were identified in the subsurface samples RB1 and RB2 respectively (Fig. 1). However, the lowest variability (4 genera) was unexpectedly observed in the soil P1 sample (Fig. 3). We need to take into consideration the fact that until today it is not entirely clear how accurately cultivable microorganisms represent the overall microbial diversity in the environment. The culture-based approach is limited as most of these microorganisms cannot be cultivated under laboratory conditions (Štursa et al., 2009). The overall abundance of cultivable microorganisms in the studied samples was also interesting, with hundreds of thousands of CFU (colony forming units) in 1 gram of sample (from $2,18 \times 10^5$ CFU per 1.0 gram of the RB1 sample to $4,51 \times 10^5$ per 1.0 gram of the P1 sample).

The presence of several bacterial genera identified in the samples from Rozália mine and mine-associated sites

was also observed in other studies dealing with the microbial diversity of the gold mine environment or the biogeochemical cycle of gold. For example, the genus *Paenibacillus* which was found in the sample collected from the deep subsurface (1.5 km depth) of the Homestake gold mine in Lead, South Dakota, USA (Rastogi et al., 2009), other genera such as *Arthrobacter*, *Microbacterium*, *Micrococcus*, *Pseudomonas* and *Sphingomonas* represented a significant proportion of the identified taxa in the samples from the gold mine in Złoty Stok, Poland (Drewniak et al., 2008). Our results also show an overlap with other work in identifying the presence of the genera *Acidovorax*, *Acinetobacter*, *Hydrogenophaga*, *Micrococcus*, *Pseudomonas*, *Rhizobium*, *Sphingomonas* and *Staphylococcus* in biofilms on Au grains and nuggets from Australia, New Zealand and South America (Rea et al., 2016).

The mechanisms of participating in the biogeochemical cycle of gold have already been described in several bacterial genera which have also been identified in our samples (Tab. 1).

Conclusion

The exploration of gold mine-associated autochthonous microbiota and its biogeochemical potential provides new findings and possibilities in the field of precious metals microbial biominerology. Our work deals with study of bacteria inhabiting deep subsurface environment of Rozália gold mine in Hodruša-Hámre and above-ground sites associated with mine. Although these locations represent a certain type of extreme environments our results demonstrate the presence of a relatively diverse bacterial population. Using combination of culture-based and molecular methods, 18 different bacterial genera were identified in the examined samples. The role of several of these genera in the biogeochemical cycle of gold has already been reported. The real participation of our bacterial isolates in the individual steps of gold cycle and their potential in biominerology will be the subject of subsequent experiments.

Acknowledgements

The work was financially supported by the Slovak Grant Agency for Science VEGA grant no. 1/0229/17 and by the The Slovak Research and Development Agency grant no. APVV SK-PL-18-0012.

Literatura – References

1. BAUER, V. et al. Present state of ore mining in Slovak republic. In *Acta Metallurgica Slovaca*, 1, 2002, p. 59–68, ISSN 1338–1156.
2. DREWNIAK, L. et al. Bacteria, hypertolerant to arsenic in the rocks of an ancient gold mine, and their potential role in dissemination arsenic pollution. In *Environmental pollution*. 156(3), 2008, p. 1069–1074. ISSN 0269–7491.
3. EHRLICH, H., NEWMANN, D. *Geomicrobiology*. 5th edition. Boca Raton : CRC Press, 2009. p. 628. ISBN 978-0-8493-7906-2.
4. GADD, G. M. Microbial Role in Global Biogeochemical Cycling of Metals and Metalloids at the Interfaces in the Earth's Critical Zone. In: XU J., HUANG P. M. (eds) *Molecular Environmental Soil Science at the Interfaces in the Earth's Critical Zone*. Berlin, Heidelberg : Springer, 2010, p. 5–7. ISBN 978-3-642-05296-5.
5. KUBAČ, A. et al. Mineralogy of the epithermal precious and base metal deposit Banská Hodruša at the Rozália Mine (Slovakia). In *Mineralogy and Petrology*. 112(5), 2018, p. 705–731. ISSN 1438–1168.
6. LENGKE, M. et al. Mechanisms of gold bioaccumulation by filamentous cyanobacteria from gold(III)-chloride complex. In *Environmental Science & Technology*. 40(20), 2006, p. 6304–6309. ISSN 1520–5851.
7. PAL, A., PAUL A. Microbial extracellular polymeric substances: central elements in heavy metal bioremediation. In *Indian journal of microbiology*. 48(1), 2008, p. 49–64. ISSN 0973–7715.
8. RASTOGI, G. et al. Isolation and characterization of cellulose-degrading bacteria from deep subsurface of the Homestake gold mine, Lead, South Dakota, USA. In *Journal of industrial microbiology and biotechnology*. 36(4), 2009, p. 585–598. ISSN 1476–5535.
9. REA, M. et al. 2016. Bacterial biofilms on gold grains – implication for geomicrobial transformations of gold. In *FEMS Microbiology Ecology*. 92(6), 2016, fiw082, ISSN 0168–6496.
10. REITH, F. et al. 2006. Biomineralization of gold: Biofilms on bacterioform gold. In *Science*, 303(5784), 2006, p. 233–235. ISSN 1095–9203.
11. SHUSTER, J., REITH, F. Reflecting on Gold Geomicrobiology Research: Thoughts and Considerations for Future Endeavors. In *Minerals*, 8(9), 2018, p. 401. ISSN 2075–163X.
12. ŠTURSA, P. et al. Approaches for diversity analysis of cultivable and non-cultivable bacteria in real soil. In *Plant Soil and Environment*, 55(9), 2009, p. 389–396, ISSN 1214–1178.
13. WEISBURG, W. G. et al. 16S ribosomal DNA amplification for phylogenetic study. In *Journal of bacteriology*, 173(2), 1991, p. 697–703, ISSN 1098–5530.

Ukryty mikrokosmos w słowackiej kopalni złota Rozalia – drobnoustroje górnikami złota?

Cykl biogeochemiczny złota obejmuje dyspersję i ponowne zateżnienie złota (Au) w wyniku procesów fizycznych, chemicznych i biologicznych w środowiskach powierzchni Ziemi. Procesy te są wywoływane przez aktywność metaboliczną różnych taksonów drobnoustrojowych, ale wiele z nich (a także ich potencjał biogeochemiczny) jest wciąż niezbadanych. Zrozumienie obiegu złota jest niezbędne do opracowania innowacyjnych, przyjaznych dla środowiska technik przetwarzania złota. Nasze eksperymenty miały na celu izolację i identyfikację bakterii heterotroficznych z próbek rudy i składowiska rudy zebranych w kopalni złota Rozália w Hodruša-Hámre. Stosując podejście oparte na hodowli, a następnie połączenie profilowania białka MALDI-TOF MS i sekwencjonowania 16S rDNA, zidentyfikowano 18 różnych rodzajów bakterii w badanej mikroflorze. Stwierdzono udział kilku przedstawicieli tych rodzajów w poszczególnych etapach złotego cyklu. Rzeczywiste zaangażowanie izolatów bakteryjnych w reakcje transformacji złota i ich potencjał biogeochemiczny zostaną zbadane w kolejnych eksperymentach.

Słowa kluczowe: kopalnia złota, cykl biogeochemiczny, różnorodność bakterii