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 subjective 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 Rozália 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
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 μL aliquots of all dilutions were spread in parallels on four types of culture media (TSA – Trypticase Soy Agar, NA2 – Nutrient Agar no. 2, 10×NA2 – 10 × 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,
Germany) was used. Each PCR reaction mixture (50 μL) contained 1 μM of D1 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 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 biomining. 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 biomining will be the subject of subsequent experiments.

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| Bacterial genus | Potential effect on Au-cycling process | Reference |
|----------------|----------------------------------------|-----------|
| _Arthrobacter_  
_Microbacterium_  
_Micrococcus_  
_Staphylococcus_ | Mediation of initial colonization of the Au-grains surface | Rea et al., 2016 |
| _Acinetobacter_  
_Pseudomonas_ | Biofilm growth and recruitment | Pal and Paul, 2008 |
| _Rhizobium_  
_Acidovorax_  
_Pseudomonas_ | N₂ cycling and continuous supply of usable nitrogen to other biofilm organisms | Rea et al., 2016 |
| _Acinetobacter_  
_Arthrobacter_  
_Pseudomonas_ | Metabolic turnover of complex organics, xenobiotic and toxins | Rea et al., 2016 |
| _Pseudomonas_ | Au solubilization  
Precipitation of gold colloids | Reith et al., 2006 |

Tab. 1. Bacterial genera with proven participation in the biogeochemical cycle of gold

Tab. 1. Rodzaje bakterii o udowodnionym udziale w biogeochemicznym cyklu złota
Ukryty mikrokosmos w słowackiej kopalni złota Rozalia – drobnoustroje górnikami złota?

Cykl biogeochemiczny złota obejmuje dyspersję i ponowne zatężanie 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 heterotроficznych z próbek rudy i składowiska rudy zebranych w kopalni złota Rozalia 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 eksperymencach.

**Słowa kluczowe:** kopalnia złota, cykl biogeochemiczny, różnorodność bakterii

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