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Bacteria diversity and arsenic mobilization in rock biofilm from an ancient gold and arsenic mine

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HIGHLIGHTS

- Mine biofilm consortium capable to mobilize arsenic from rock
- Fairly high biofilm diversity with high number of α-Proteobacteria
- Evidence for potential role of this biofilm in dissemination of arsenic

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ABSTRACT

In this paper we characterize the biofilm community from an ancient Złoty Stok gold and arsenic mine. Bacterial diversity was examined using a culture-independent technique based on 16S rRNA gene amplification, cloning and sequencing. We show that unexpectedly the microbial diversity of this community was extremely high (more than 190 OTUs detected), with the most numerous members from *Rhizobiales* (*α-Proteobacteria*). Although the level of rock biofilm diversity was similar to the microbial mat community we have previously characterized in the same adit, its taxonomic composition was completely different.
Detailed analysis of functional *arrA* and *aioA* genes, chemical properties of siderophores found in pore water as well as the biofilm chemical composition suggest that the biofilm community contributes to arsenic pollution of surrounding water in a biogeochemical cycle similar to the one observed in bacterial mats. To interpret our results concerning the biological arsenic cycle, we applied the theory of ecological pyramids of Charles Elton.

1. Introduction

One of the greatest threats to civilization of the modern world is lack of access to clean drinking water. According to recent data (2012) of the World Health Organization (WHO) about 900 million people (roughly one-eighth of the world's population) are deprived of safe drinking water (World Health Organization).

Among the many factors reducing the quality of drinking water microbial and chemical pollution are the most dominant. One of the most toxic elements naturally occurring in the environment is arsenic. This metalloid is widely distributed in the Earth crust (Wedepohl, 1991) and constitutes a public health threat in many parts of the world. The problem of drinking water contamination by arsenic also affects Poland. Raised levels of arsenic (up to 26 mg/l) have been observed in the "Toxic" stream, the common customary name of the Gold stream (Złoty Potok) that flows out of the closed, ancient Złoty Stok gold mine located in Lower Silesia. Most of the galleries and shafts (covering about 200 km) of the mine are filled with water and are partly or totally inaccessible. Interestingly, the main source of water, except rain water, that augments the mine system is the Gold stream, which in its stretch before the mine area contains trace concentration of arsenic (below 10 μg/l). Thus, the obvious conclusion that the main source of pollution of the "Toxic" stream is the mine, which holds significant (the largest in Poland) deposits of arsenic minerals (arsenopyrites and lollingite) (Muszer, 2011).

It is commonly known that microorganisms play a key role in mobilization of heavy metals from their deposits. Microbes may dissolve and use different minerals as a source of nutrients or substrates for respiration. The most important microorganisms that play a key role in the biogeochemistry of arsenic are chemolithoautotrophs, which can use primary arsenic minerals (like arsenopyrite) as a source of energy, as electron donors. Among them, we can distinguish arsenite oxidizing bacteria, which directly transform and release arsenic (Rhine et al.,

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2008; Lebrun et al., 2003), and sulphur and iron oxidizing microbes that can indirectly remove arsenic from iron/sulfide minerals (Chen et al., 2011). Also an important group of arsenic metabolizing microbes is constituted by bacteria capable of dissimilatory reduction. They can use arsenic or associated heavy metals (e.g. iron), mainly from secondary minerals, as an electron acceptor in respiratory processes (Newman et al., 1997). In addition to microorganisms that catalyze redox processes, a relevant group of microbes is arsenic-resistant prokaryotes, which dissolve minerals to acquire nutrients. They produce organic acids or specific organic ligands (like siderophores that form strong complexes with metals), which support arsenic mineral dissolution (Gadd, 2010).

Two types of prokaryotic communities colonize the end section of the Gertruda Adit in the Złoty Stok mine: mats in the bottom sediments (Drewniak et al., 2012) and a thick slimy biofilm on the mine walls (Fig. 1). We were interested to know whether those microbes have an impact on the biogeochemical cycle of arsenic in the mine environment, and consequently, contribute to the dissemination of arsenic contamination. In our previous papers, we characterized bacteria isolated from the Złoty Stok mine rock biofilms (Drewniak et al., 2008a, 2010) and microbial mats from the bottom sediments (Drewniak et al., 2008b, 2010). Surprisingly, arsenite oxidizers and dissimilatory arsenate reducers were isolated only from microbial mats (Drewniak et al., 2008b). No chemolithoautotrophs, which could use arsenite as an electron donor, as well as arsenate — as an electron acceptor, were isolated from the rock biofilms. However, as shown by our previous studies on microbial mat community using uncultured methods (Drewniak et al., 2012), the structure and composition of the microbial community may be quite different from the one determined based on culture methods. The 16S rRNA sequence-based analysis revealed an extremely high biodiversity of the mat community able to transform ferrous arsenate minerals,

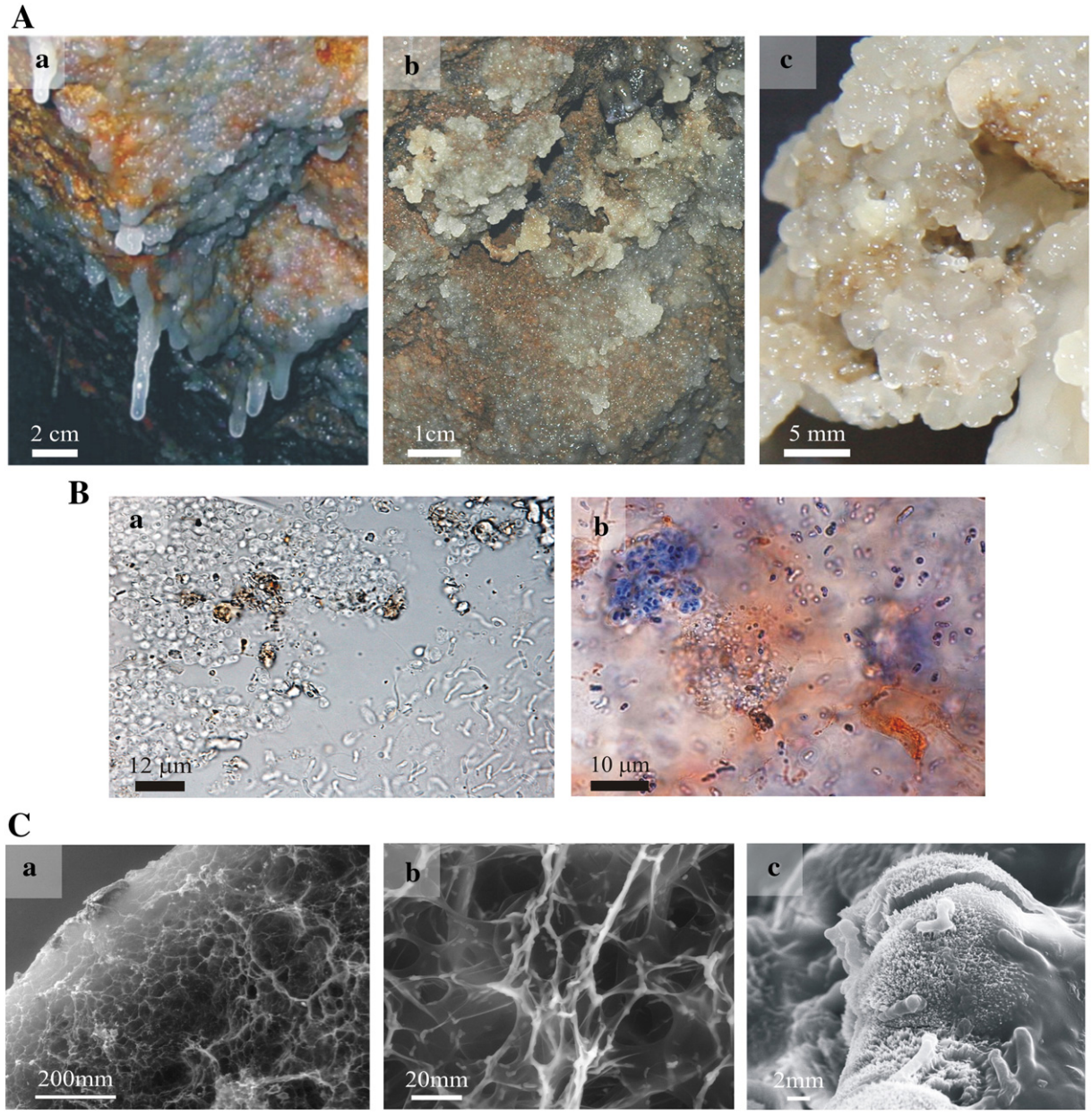

Fig. 1. Rock biofilm in the Złoty Stok gold mine. (A) Rock walls in the Gertruda Adit covered by the biofilm (a,b) and a close-up (c) photograph of the biofilm. (B) a — micrograph of unstained biofilm in DIC; b — micrograph of eosin Y-stained biofilm, showing agglomerations of microorganisms in an extracellular matrix. (C) Scanning electron micrograph of an unprocessed sample of the biofilm: a, b — magnification 150× and 2000×, respectively; c — scanning electron micrograph of a dried and fixed biofilm sample, magnification 8000×.