ELSEVIER

Institut Pasteur

Research in Microbiology xx (2014) 1–6

www.elsevier.com/locate/resmic

Microbial diversity at the moderate acidic stage in three different sulfidic mine tailings dumps generating acid mine drainage

Q3 Hananeh Korehi, Marco Blöthe, Axel Schippers*

Geomicrobiology Unit, Federal Institute for Geosciences and Natural Resources (BGR), Stilleweg 2, D-30655 Hannover, Germany

Received 14 April 2014; accepted 30 August 2014

Abstract

In freshly deposited sulfidic mine tailings the pH is alkaline or circumneutral. Due to pyrite or pyrrhotite oxidation the pH is dropping over time to pH values <3 at which acidophilic iron- and sulfur-oxidizing prokaryotes prevail and accelerate the oxidation processes, well described for several mine waste sites. The microbial communities at the moderate acidic stage in mine tailings are only scarcely studied. Here we investigated the microbial diversity via 16S rRNA gene sequence analysis in eight samples (pH range 3.2–6.5) from three different sulfidic mine tailings dumps in Botswana, Germany and Sweden. In total 701 partial 16S rRNA gene sequences revealed a divergent microbial community between the three sites and at different tailings depths. Proteobacteria and Firmicutes were overall the most abundant phyla in the clone libraries. Acidobacteria, Actinobacteria, Bacteroidetes, and Nitrospira occurred less frequently. The found microbial communities were completely different to microbial communities in tailings at <pH 3 described in the literature.

Q1 *Keywords:* Mine tailings; Pyrite oxidation; Acid mine drainage; Acidophiles; Microbial diversity; 16S rRNA gene sequencing; Firmicutes; Proteobacteria

1. Introduction

Mine tailings are fine-grained waste left over from the metal extraction process. This material often contains several percent of metal sulfides, i.e. pyrite (FeS_2) or pyrrhotite ($Fe_{1-x}S$, with $x = 0–0.125$). In freshly deposited sulfidic mine tailings the pH is alkaline or circumneutral. Due to exposing the tailings material to air and water, the metal sulfides get oxidized and the pH is dropping over time to low values, and acid mine drainage is generated. Over time an acidic oxidized zone enriched in secondary minerals such as iron(III)hydroxides develops above an unoxidized zone with unaltered material in tailings dumps [1–4].

The metal sulfide oxidation process is dramatically accelerated by iron- and sulfur-oxidizing prokaryotes at pH values below 3, at which ferric iron remains in solution and can serve as efficient oxidant for the metal sulfides, thereby being reduced to ferrous iron. The ferrous iron is oxidized to ferric iron by acidophilic iron-oxidizers such as *Acidithiobacillus* spp., *Leptosprillum* spp., *Ferroplasma* spp., '*Ferrovum*' spp. *Alicyclobacillus* spp. and *Sulfobacillus* spp. [5,6]. Acidophilic iron-oxidizers are regularly found in acid mine drainage [7–11], in bioleaching operations [6,12], in acidic abandoned mines [13,14] as well as in mine tailings at low pH [4].

The microbial diversity in several sulfidic mine tailings has been studied based on cultivation approaches [1,15–21] as well as by 16S rRNA gene sequencing [20,22–29]. Previous tailings studies revealed the predominance of iron- and sulfur-oxidizing acidophiles at low pH, but microbial communities at the moderately acidic oxidation stage (between the initial circumneutral to alkaline pH and the strong acidic final stage) have only been studied for mine tailings sites in China [24,26–28].

For further exploration of these communities at different geochemical tailings properties and climatic conditions we

* Corresponding author.
E-mail addresses: hananeh.korehi@bgr.de (H. Korehi), marco.bloethe@bgr.de (M. Blöthe), axel.schippers@bgr.de (A. Schippers).

http://dx.doi.org/10.1016/j.resmic.2014.08.007
0923-2508/

investigated the microbial diversity via 16S rRNA gene sequence analysis in eight samples in the pH range 3.2–6.5 from three different sulfidic mine tailings dumps located in Botswana, Germany and Sweden. Quantitative microbial community analysis of the same acid mine drainage generating tailings focused previously on the acidophiles *Acidithiobacillus* spp., *Leptosprillum* spp. and *Sulfobacillus* spp. [1].

2. Materials and methods

2.1. Field site description

Samples from different tailings depths were collected between 2003 and 2006 from three sulfidic mine tailings dumps near Selebi-Phikwe (Botswana), Freiberg (Germany), and Kristineberg (Sweden), as described previously [1,19,21,30,31]. The uncovered and active tailings dump near Selebi-Phikwe contained the reactive pyrrhotite as the main metal sulfide. Alternating grey and brown layers were found throughout the whole tailings depth profile. The brown color originated from precipitation of iron(III)hydroxides. The climate is semi-arid with an average annual temperature of 21 °C [30]. The climate at the covered pyrite-containing tailings dump at Kristineberg is cold and humid with an annual average temperature of 0.7 °C. Before covering in 1996 with about 2 m thick till, pyrite oxidation led to a pronounced about 0.5 m thick brown oxidized zone [31]. The uncovered and inactive tailings located in Freiberg at temperate climate with an annual average temperature of 7.7 °C contained as main metal sulfides pyrite, arsenopyrite, sphalerite, and galena. Due to vertical metal transport in the oxidized zone and metal precipitation, a brown hardpan formed at a distinct depth within the tailings [19].

Eight samples from different sampling depths from the three tailings in the pH range 3.2–6.5 were selected for 16S rRNA gene cloning and sequencing. Geochemical and microbial data for the eight selected samples obtained in previous studies are presented in Table 1. The microbial community was dominated by *Bacteria*, the *Archaea* and *Eukarya* played only a minor role in all tailings [1]. Thus, our diversity analysis focused on *Bacteria*.

2.2. DNA extraction and 16S rRNA gene library construction

The extraction of genomic DNA was performed with 0.5 g of a frozen tailings sample using the FastDNA Spin Kit for soil (Bio 101) protocol. Community 16S rRNA genes were amplified by PCR with the universal bacterial primer pairs GM3f (5′ AGA GTT TGA TCA TGG C 3′) and GM4r (5′ TAC CTT GTT ACG ACT T 3′) [32] for *Bacteria* in 50 µl final volume. PCR mixture was prepared from Thermo Scientific MasterMix (final concentration: 75 mM Tris-HCL (pH 8.8), 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.5 µM of each primer, 0.652 U ThermoPrime Tag DNA polymerase, 100 ng/µL BSA) and 2 µL template of extracted DNA. Negative controls without template were used as a contamination check. Amplification reactions occurred during 35 cycles and included denaturation at 95 °C for 45 s, extension at 72 °C for 90 s and annealing at 55 °C for 30 s. Afterwards the amplicons of PCR reactions were commercially cloned and sequenced by DNA Sanger sequencing, LGC Genomics, Berlin, Germany. Overlapping sequencing from both sides of the 16S rRNA genes was performed. Contigs were constructed with the software Geneious Pro 5.4 and were aligned and checked for chimeric artifacts with UCHIME implemented in the Mothur v1.30 program package [33] and discarded if discovered. In total 701 partial 16S rRNA gene fragments were obtained after quality-, alignment-, and chimera-check for subsequent analysis. The obtained 16S rRNA gene sequences were assigned to 105 operational taxonomic units (OTU, 97% similarity) and representative sequences from each OTU were selected by the average distance criteria. The representative OTU sequences were taxonomically assigned using the SINA online aligner with the ARB sequences database SSURef_NR99_117 as template. For phylogenetic analysis the sequences alignment was curated by hand with the ARB software package (v.5.4) [34]. Sequences that could not be aligned reliable were discarded from the phylogenetic tree construction. For phylogenetic tree construction selected reference sequences (ten for each OTU) together with the OTU-representatives were used for maximum likelihood algorithm (RAxML) with GTRGAMMA as rate distribution model [35] available from the CIPRES Science Gateway [36], the general bacteria filter provided in ARB, and a bootstrap test with 1000 replicates. The 16S rRNA gene sequence from *Nitrosopumilus maritimus* SCM1 (CP000866) was used as an out-group. The 16S rRNA gene sequences obtained in this study were submitted to GenBank with the accession numbers KJ650684–KJ650788. Q2

3. Results and discussion

The microbial communities in three different sulfidic mine tailings dumps at pH 3.2 to 6.5 were identified by 16S rRNA gene sequencing analysis and the overall results revealed a strong differentiation of the microbial composition with depth as well as between the three tailings. On the one hand the microbial community composition shifts since the tailings dumps samples were in different acidic stages due to ongoing oxidation processes. On the other hand the activity of microorganisms altered the geochemical conditions of the tailings dump that resulted in a high abundance of acidophilic iron- and sulfur-oxidizing chemolithotrophs at the final weathering stage [1]. In previous investigations the role of pH as most important factor for the microbial community composition and the geochemistry was determined in tailings field site studies [24,26–28] as well as in laboratory experiments [37,38].

The taxonomic classification of the obtained bacterial 16S rRNA gene sequences in this study clustered in the six phyla *Proteobacteria*, *Firmicutes*, *Nitrospirae*, *Actinobacteria*, *Acidobacteria*, and *Bacteroidetes*. The most abundant OTUs contained representatives of *Firmicutes* and *Proteobacteria* (Table 2). The two samples with pH 6.4 (S1) and 6.5 (K1) at the uppermost sampling depth exhibited a much higher

Please cite this article in press as: Korehi H, et al., Microbial diversity at the moderate acidic stage in three different sulfidic mine tailings dumps generating acid mine drainage, Research in Microbiology (2014), http://dx.doi.org/10.1016/j.resmic.2014.08.007