Investigating Manganese-oxidizing microbial Biofilms in a historic Copper Mine of Upper Frankonia

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Abstract

Providing evidence for presumed chemolithoautotrophic manganese oxidation remains a major and challenging objective in subsurface microbiology. Here, we report on the dissection of blackish, leathery microbial biofilms discovered in the “Goldene Falk”, a historic copper mine in Northern Bavaria, with mine shafts originating back to the 15th century. Biogeochemical analysis of the biofilm indicated a notable enrichment of manganese oxides (MnOX), with Mn making up for more than 10% (dry weight) of the deposits. STXM analysis suggested a clear biogenic origin of MnOX in situ. Characteristic nodules of MnOX with microbial cells attached were also found in aerobic Mn-oxidizing enrichment cultures set up in minimal media in the lab. The biofilms obtained from the mine were also subjected to amplicon and metagenomic sequencing, revealing a vast diversity of presumably chemolithoautotrophic and heterotrophic microbial lineages, including members of the Pyrinomonadaceae, Rhizobiales, Methylomirabilaceae and also lineages within the Nitrospiraceae previously reported to be associated with lithotrophic Mn oxidation. We reconstructed >100 high-quality bacterial genomes (MAGs), many of them carrying genomic signatures of biogenic Mn oxidation (albeit non-lithotrophic). We continue to investigate the biofilms, our enrichment cultures and the metagenomic data obtained from the mine for further evidence of possible autotrophic manganese oxidation, the macroscopic leathery biofilm representing a likely habitat for these still enigmatic microbes.
Indications for nitrogen and sulfur cycling also ongoing in the biofilms will also be discussed. This research contributes to a better understanding of the yet-enigmatic capacities of the microbiota in man-made subsurface environments.

**Keywords**
Cave and mine microbiology, subsurface biofilms, chemolithoautotrophy, biogeochemical Mn cycling, metagenomics

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**Conflicts of interest**
The authors have declared that no competing interests exist.