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Heavy metal resistance mechanisms in actinobacteria for survival in AMD contaminated soils

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Abstract

A site in the former uranium mining area of Eastern Thuringia near Ronneburg was investigated with regard to effects of acid mine drainage (AMD) on reactive transport and bioattenuation. Processes involved in this attenuation might include physico-chemical reactions in reactive transport as well as activities of microorganisms for bioattenuation. In order to test the influence of the soil microbes, a mapping was carried out including both hydrogeochemical and microbiological parameters.

Mapping of contamination was performed along the banks of a creek in a 900 m stretch in 50 m steps by hydrogeochemical analysis of water extracts of soil samples, while general microbial activity was scored by examining soil respiration. The soil samples with high heavy metal load did show low soil respiration as a parameter for microbial activity and plating revealed minimal counts for spore producing bacteria at these contaminated locations. Actinobacteria strains isolated from adjacent locations revealed high levels of resistance as well as high numbers of resistant strains. Specific responses in actinobacteria were investigated after isolation from each of the 18 measuring points along the creek. Specific adaptation strategies and high yields of (intra)cellular heavy metal retention could be seen. Several

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strategies for coping with the high heavy metal contents are further discussed and genes for proteins expressed specifically under high nickel concentration were identified by two-dimensional gel electrophoresis.

Keywords: AMD; Actinobacteria; Streptomycetes; Heavy metal resistance; Bioremediation; Soil extract; Bioavailable heavy metals; Proteome analysis; Molecular biology

1. Introduction

Bacteria in soil consist of different taxa, among them aerobic, spore forming, Gram-positive bacteria of the *Bacillus* and actinobacteria groups. Spore formation is thought to be an adaptation to the differing conditions in soil with changing water contents due to dryness and rain (Vobis, 1997). Actinobacteria including streptomycetes are known to be prominent representing up to 20% of the aerobic soil bacteria population in arable land, and their strong secondary metabolism makes them good candidates for identification of components altering soil chemistry. This capacity of formation of secondary metabolites is exemplified by production of geosmin, the compound responsible for the odor of tilled soil, as well as by production of antibiotics (Kieser et al., 2000). Other products of secondary metabolism may enable the bacteria to cope with stress factors including toxic levels of heavy metals (So et al., 2000).

While many heavy metals are essential micronutrients since they are incorporated into enzymes and cofactors (Fe, Zn, Mn, Co, Cu, Ni, V, Mo) they still are toxic in high concentrations because of adversary binding to enzymes and DNA, and by production of oxygen radicals through the Fenton reaction (Lopez-Maury et al., 2002). Therefore, the organisms must maintain a homeostasis within the cell that keeps the reactive heavy metals at an optimal, sub-toxic level. Resistance factors may allow them to maintain intracellular low levels of heavy metals or intracellular fractionation of the metal in non-harmful complexes (Eitinger and Mandrand-Berthelot, 2000). Thus, adapted microbial populations are prone to show higher resistance to heavy metals as compared to populations of non-contaminated sites.

Adaptive responses towards heavy metal stress may involve detoxification of oxygen radicals, as has been shown for superoxide dismutase overproducing strains of the bacterium *Escherichia coli* which are tolerant to higher heavy metal concentrations (Geslin et al., 2001). Streptomycetes are known to possess two superoxide dismutases: one iron- and one nickel-containing enzyme which are regulated by nickel (Kim et al., 1998a, b). Other resistance mechanisms include sequestration of heavy metals and adsorption of generally positively charged heavy metal cations to the cells walls. The cell walls of the Gram-positive bacterium *Bacillus sphaericus* are commercially used for water treatment (Beveridge and Murray, 1976; Doyle et al., 1980; Raff et al., 2003). In addition, active uptake of radioisotopes has been known at least since the Chernobyl fall-out for basidiomycete fungi which led to extremely high enrichment of cesium in fruitbodies