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Growth of streptomycetes in soil and their impact on bioremediation

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HIGHLIGHTS

- The decrease of metal bioavailability in soil that contains streptomycetes is measured.
- Plant growth improvement is seen by inoculation with selected strains.
- Metal resistant strains were used for altering metal mobility in soil and plant growth promotion.

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ABSTRACT

The impact of the extremely heavy metal resistant actinomycete *Streptomyces mirabilis* P16B-1 on heavy metal mobilization/stabilization, phytoremediation and stress level of plants was analyzed in the presence and absence of *Sorghum bicolor* in sterile microcosms containing highly metal contaminated or control soil. For control, a metal sensitive *S. lividans* TK24 was used. The metal contents with respect to the mobile and specifically adsorbed fractions of the contaminated soil were considerably decreased by addition of both, living and dead biomass of the strains, with the heavy metal resistant *S. mirabilis* P16B-1 showing considerably higher impact. Both strains could grow in control soil, while only *S. mirabilis* P16B-1 formed new tip growth in the metal contaminated soil. A plant growth promoting effect was visible for *S. mirabilis* P16B-1 in contaminated soil enhancing the dry weight of inoculated *Sorghum* plants. Thus, metal resistant strains like *S. mirabilis* P16B-1 are able to enhance phytoremediation of heavy metal contaminated soils.

1. Introduction

Soils are the basis as well as main organizers of terrestrial ecosystems containing mineral components and organic matter, with microorganisms being involved in both soil formation and soil function. These interactions are based on microbially enhanced or controlled reactions like formation of metal oxides, mineral formation, formation of humic substances, enzyme stabilization, aggregate formation, turnover and cycling of C, N, P and S, as well as the fate and transformation of inorganic and organic pollutants [1]. Drivers of microbial processes are energy input, moisture, solid composition and composition of organic matter (soil organic matter; SOM), making the formation of soil structures highly dynamic [2] and scale-dependent [3]. This heterogeneity clearly hampers investigations under natural conditions.

Heavy metal contamination in pyrite-rich soils is often found as a result of oxygen introduction through mining activities resulting in acid mine drainage formation, which not only has a toxic effect on microorganisms and plants, but also affects SOM formation and turnover [4–6]. The release of rare earth elements (REE), other heavy metals and arsenic, as well as acid and sulphate production, are characteristic [4,7,8] and have become an economic and environmental problem in (former) mining areas world-wide [9]. A cost-efficient strategy to treat heavy metal contaminated areas is phytoremediation [10,11]. In addition, it provides a vegetation cover preventing wind and water erosion [12].

The use of heavy metal resistant soil bacteria and fungi, rhizosphere bacteria, or mycorrhizal fungi, i.e. microbially enhanced phytoremediation, has been shown to have a particularly positive effect on plant performance [7,13–16]. However, the mechanisms of the related microbial action(s) are not fully understood, but may include alteration of chemical properties and control of metal

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availability as well as enhancing plant growth and modifying plant metal uptake [17].

Sorghum bicolor is known for its capacity to accumulate several metals and being drought resistant [18–22]. Harvested plant biomass is suitable for production of bioethanol [23] which allows for land-use of metal contaminated soils without threatening food chains, nor competing with food production. However, metal contamination may limit usage. Most studies on microbially enhanced *Sorghum* remediation reported mycorrhizal symbiosis with *Glomus* [24–26] or (co-)inoculation with rhizobacteria [27,28]. Among the bacteria used, Gram-positive, filamentous streptomycetes are prominent [29].

Here, we developed an axenic microcosm system to study streptomycetes' impact on heavy metal mobility, phytoremediation and growth of *Sorghum*. The presented data provide an insight into the role of streptomycetes in bioremediation and may help to understand the interaction between microorganisms and plants with special emphasis on heavy metal contamination in soil.

2. Experimental

2.1. Strains and culture conditions

S. mirabilis P16B-1 [30] is known to grow directly in heavy metal contaminated soil without addition of media components [29]. As metal sensitive control, *S. lividans* TK24 was used [31,32]. Biomass was grown in 7 l fermenters (BIOSTAT® B-DCUII, Sartorius Stedim Biotech) with 5 l medium (glucose monohydrate 5 g/l, soluble starch 25 g/l, casein–peptone 10 g/l, yeast extract 5 g/l, $(NH_4)_2SO_4$ 1.5 g/l, KH_2PO_4 1.5 g/l, $CaCO_3$ 5 g/l, trace element solution 1 ml ($ZnCl_2$ 40 mg/l, $FeCl_3 \times 6\ H_2O$ 200 mg/l, $CuCl_3 \times 6\ H_2O$ 10 mg/l, $MnCl_2 \times 4\ H_2O$ 10 mg/l, $Na_2B_4O_7$ 10 mg/l, $(NH_4)_2Mo_7O_{24} \times 6\ H_2O$ 10 mg/l) pH 7.0) at 24 °C, 400 U/min and 600 U/min after 24 h, pO_2 > 20%, aeration 2 slpm and pH > 6.0 controlled by addition of 10% NaOH. After growth, mycelium was harvested by centrifugation and washed two times with tap water (Megafuge, 4000 rpm, 15 min, 20 °C). Dead biomass was obtained by autoclaving 20 min at 121 °C.

2.2. Soil samples and analyses

Soil sample M (heavy metal contaminated) was collected at a former uranium mining site, Ronneburg, Germany, from the banks of the creek Gessenbach at sample site K7 [33]. Control soil C was obtained from the municipal park 'Paradies' in Jena, Germany (GPS 44709773, GK 5642870). The soil was dried at 30 °C and sieved to a maximum grain size of 2 mm. Gamma radiation (min. dose 78 kGy, max. dose 90 kGy) was used to sterilize the soil (Synergy Health Radeberg, Germany) which was controlled by plating. The water holding capacity (WHC) was measured using 100 g of soil in a glass drip dowsed with 100 ml of distilled water and discharge was quantified after 24 h.

Sequential extraction [34] and analysis of extraction solutions (F1–F6) were used to characterize metal contents (ICP-MS, XSeries II, ThermoFisher Scientific, Bremen, Germany). Mobile (F1), specifically adsorbed (F2), metals bound to Mn oxides (F3), to organic matter (F4), to poorly crystalline Fe oxides (F5) and to crystalline oxides (F6) were compared to total contents measured after extraction with HF and $HClO_4$. Bioavailable metal contents were defined as loads in mobile (F1) and specifically adsorbed (F2) fractions.

2.3. Normalization and statistical treatment

Three replicates were used for all soil samples. Alteration of metal contents in F1 and F2 was obtained by normalization of values to metal loads at day 0. Levene' test (*F*-test) was used to assess the equality of variances in different samples. *T*-test was applied to identify statistically significant differences between time points with alpha level set at 0.05. Additional values for REE (day 30) have been normalized to Post Archean Australian Shale [35].

2.4. Experimental setup of microcosms and phyto-tubes

Thirty grams of soil (M or C) were transferred into sterile tubes (Greiner Bio One, 100 ml screw top), inoculated with 0.648 g of fresh or autoclaved bacterial biomass and set to a water holding capacity of 70% by addition of sterile tap water. Phyto-tubes (Roth, 30 mm in diameter, 200 mm in height, sealed with sterile plugs) were planted with *Sorghum bicolor* seeds (one per tube) pre-sterilized for 30 min using 30% hydrogen peroxide and pre-germinated for 48 h at 23 °C. Microcosms and phyto-tubes were incubated in a climate chamber with a day/night rhythm of 23/18 °C and 70% humidity for 30 d.

2.5. Re-isolation of strains from soil

The inoculated strains were re-isolated from soil suspensions 1:10 in 0.9% NaCl after incubation on a rotary shaker for 24 h (180 rpm, 28 °C), centrifugation (Megafuge 3000 rpm, 1 min) and plating (10^{-1} to 10^{-5}) onto AM medium containing cycloheximide (1 mM). After 6 days of incubation at 28 °C, colonies were transferred to complex TSB medium (Difca, Heidelberg) for efficient biomass production (6 days, 28 °C). DNA was isolated by 12 freeze thaw cycles [30] and used for PCR to amplify 16S rDNA (primers: 16SfD1: 5′-AGAGTTTGATCCTGGCTCAG-3′, 16SrP2: 5′-ACGGCTACCTTGTTACGACTT-3′; PCR program: 94 °C, 10 min; 30 cycles: 94 °C, 60 s; 56 °C, 30 s; 72 °C, 90 s; 72 °C, 10 min, Thermocycler Biorad). Sequencing of the resulting PCR product was performed by GATC Biotech (Konstanz, Germany).

2.6. Plant assessment and fluorescence measurements

For plant assessment, height of shoots, total length of leaves (sum of all lengths of leaves from a single plant), number of leaves and dry mass of shoots have been determined. Additionally, chlorosis, if present, and development of roots were recorded. Dry shoot biomass of five plants was pooled (5 plants per pool, 2 replicates), ground and extracted by HF and $HClO_4$ using microwave digestion. Metal content was measured by mass spectrometry (ICP-MS, XSeries II, ThermoFisher Scientific, Bremen, Germany).

Chlorophyll fluorescences were measured to monitor photosynthetic performance to conclude quantum yield of PSII (Y) and stress level of *Sorghum* plants. The youngest, full-grown leave of a plant was cut and put on wet tissue to avoid drying. Subsequently, dark adaption (15 min) and measurements with green light (FLUORCAM 700MF; for 3 s zero fluorescence (F_0)), saturating flash of light (2000 μE), no light (60 s) and actinic light (10 min; maximum 620 nm and 90 μE; steady state fluorescence (F_t)) was measured (every 20 s). After saturated flashes of light (every 3 min), maximum fluorescence level in the light (F'_m) was determined, followed by dark (2 min) and actinic light to measure zero fluorescence (F'_0). All measurements were performed in triplicate. Quantum yield of PSII (Y), maximum quantum yield of PSII (F_v/F_m) and non-photochemical quenching (NPQ) were determined according to [36]. Additionally F_s/F_m was calculated from $(F_t - F'_0)/F_m$.

2.7. Scanning electron microscopy of soil samples

Soil was transferred into micro-porous capsules (Plano GmbH, Germany) and fixed (2% (v/v) glutaraldehyde in phosphate buffered saline (PBS buffer: NaCl 4.38 g/l, $NaH_2PO_4 \times H_2O$ 2.2 g/l, $Na_2HPO_4 \times 2H_2O$ 9.25 g/l, pH 7.2) for 1 h followed by washing