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Siderophore production by streptomycetes—stability and alteration of ferrihydroxamates in heavy metal-contaminated soil

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Abstract Heavy metal-contaminated soil derived from a former uranium mining site in Ronneburg, Germany, was used for sterile mesocosms inoculated with the extremely metal-resistant *Streptomyces mirabilis* P16B-1 or the sensitive control strain *Streptomyces lividans* TK24. The production and fate of bacterial hydroxamate siderophores in soil was analyzed, and the presence of ferrioxamines E, B, D, and G was shown. While total ferrioxamine concentrations decreased in water-treated controls after 30 days of incubation, the sustained production by the bacteria was seen. For the individual molecules, alteration between neutral and cationic forms and linearization of hydroxamates was observed for the first time. Mesocosms inoculated with biomass of either strain showed changes of siderophore contents compared with the non-treated control indicating for auto-alteration and consumption, respectively, depending on the vital bacteria present. Heat stability and structural consistency of siderophores obtained from sterile culture filtrate were shown. In addition, low recovery (32 %) from soil was shown, indicating adsorption to soil particles or soil organic matter. Fate and behavior of hydroxamate siderophores in metal-contaminated soils may affect soil properties as well as conditions for its inhabiting (micro)organisms.

Keywords Soil · Siderophores · Chelators · Ferrioxamines · Streptomycetes · Heavy metals

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Introduction

Soil (micro) organisms are essential for soil genesis and contribute to almost all chemical transformations in soil (Havlicek 2012; Verstraete and Mertens 2004). The soil network of mineral components, organic matter, organisms including plant roots, edaphone, and microbes (Cappellen 2003; Huang et al. 2005; Veis 2003) needs to be understood in all its parts to be able to address interrelations within the complex processes. This is necessary for soil maintenance or re-establishing soil quality, vitality, and usability to feed the world population and for contributing to climate, typical functions of soil (Miltner et al. 2011; O'Kane 2012).

Streptomycetes are one of the dominant groups of bacteria in soil (Streshinskaya et al. 2005), with specifically high prevalence in heavy metal-contaminated soil (Haferburg et al. 2007; Kothe et al. 2010). With their versatile secondary metabolism, streptomycetes are able to excrete a variety of low molecular weight compounds, including siderophores

(Dimkpa et al. 2008a). These chelators are well known to ascertain iron delivery to producing as well as other species to overcome the low bioavailability of iron (10^{-9}–10^{-18} M) in soils by high affinity for Fe^{III} ($Kf>10^{30}$).

Siderophores are low molecular weight compounds (500–1500 Da) and were reported to be produced by bacteria, fungi, and graminaceous plants. More than 500 different siderophores are known, approximately 270 of which have been structurally characterized (Saha et al. 2013; Hider and Kong 2010). Major siderophore classes are the hydroxamates and catecholates (Ahmed and Holmström 2014b; Hider and Kong 2010; Matsumoto et al. 2004), both being excreted by streptomycetes—even concomitantly, which may provide an advantage in competition (Chater et al. 2010). Interestingly, it could be shown that siderophore production by heavy metal-resistant streptomycetes is stimulated by other, non-Fe metals like aluminum, cadmium, copper, or nickel and that they complex an array of metals (e.g., Ni, Al, Cd, Cu, Ga, In, Pb Zn, U, and Np; see Rajkumar et al. 2010; Dimkpa et al. 2008b, 2009b). This has been discussed with respect to lowering metal ecotoxicity in addition to supporting Fe acquisition to bacteria and plants in the presence of other metals.

Since free radicals are produced chemically from heavy metals, complexing can inhibit detrimental effects of metal contamination, and plant growth-promoting molecules like auxins are protected from degradation (Dimkpa et al. 2008a, b, 2009a, b). The use of the excreted xenosiderophores supports growth in plants, even in metal-contaminated soil (Dimkpa et al. 2008a, 2009b; Vassilev et al. 2004), and other (micro)organisms. Nevertheless, understanding of actinobacterial siderophore production and their application is still fragmentary (Wang et al. 2014). Understanding the potential benefit of *Streptomyces*-derived siderophores for soil-dwelling organisms can lead to applications in bioremediation. However, this is directly dependent on the stability of the molecules and their resistance to degradation or alteration within soil. European summers with heat waves (1976, 1994, 2005) or mega heat waves (2003, 2010; Barriopedro et al. 2011; Fischer et al. 2007; Reichstein et al. 2007) may impact on thermal stability of siderophores; sparse vegetation on metal-polluted sites may increase soil temperatures additionally (Galhaut et al. 2014; Escarré et al. 2011). Here, we present methods and data to better understand the role of hydroxamate siderophores in acid mine drainage (AMD)-affected soils impacted by multi-metal contamination.

Material and methods

Soil sampling

Heavy metal-contaminated soil was collected at the sample site K7 from the AMD-affected bank of the creek Gessenbach passing through the former uranium mining site WISMUT, Ronneburg, Germany (Schmidt et al. 2005), dried at 30 °C, and sieved to a maximum grain size of 2 mm. Gamma radiation (min. 78 kGy, max. dose 90 kGy) which is reported to be the most efficient and least disruptive sterilization method (e.g., unaffecting the major mineralogy of clay, low impact on size or aggregate stability; compare Staunton et al. 2002; McNamara et al. 2003; Bank et al. 2008) was used to sterilize soil (500 g soil in 1-l Schott Duran glass bottles; Synergy Health Radeberg, Germany). Metal contents in soil were measured as bioavailable fractions (corresponding to the sum of mobile and specifically adsorbed fractions F1+F2; compare Zeien and Brümmer 1989) and total content (Grawunde et al. 2014) by inductively coupled plasma mass spectrometry (ICP-MS; XSeries II, ThermoFisher Scientific, Bremen, Germany). For total extraction, acid treatment with subsequent heat exposure (HNO_3 65 %, $HClO_4$ 70 %; ratio 3:1, incubation for 1 h at 80 °C, 1 h at 120 °C, 5 M HNO_3; filtration 0.45 μm; for details, see Grawunde et al. 2014) was performed.

Microbial inoculation

Thirty grams of soil was transferred into sterile tubes (Greiner Bio One, 100 ml with screw top), inoculated with 0.648 g of fresh bacterial biomass (for fermentation protocol and further details, see Schütze et al. 2013, 2014) of either the extremely heavy metal-resistant *Streptomyces mirabilis* P16B-1 (Schmidt et al. 2008) or the metal-sensitive control strain *Streptomyces lividans* TK24 (Amoroso et al. 2002; Ravel et al. 2000). Soil was set to a water holding capacity of 70 % by addition of sterile tap water. No nutrients were added to the soil system. Microcosms were incubated with closed lids in a climate chamber with a day/night rhythm of 23/18 °C and 70 % humidity for 30 days. The collected soil samples at 0 and 30 days were directly frozen and stored at −20 °C to avoid further siderophore production, consumption, and alteration, respectively. To prevent oxidation of molecules, the storages tubes were kept in the dark and filled completely to reduce head-space oxygen and photo-oxidation (according to suggestions of Waterman et al. 2002).

Siderophore extraction and measurement

The extraction and quantification of siderophores were performed following Ahmed and Holmström (2014a). To gain information that is independent of actual soil humidity, extraction was performed for soil-bound siderophores and siderophores present in the actual soil solution. The dissolved siderophores were extracted from 1 g of soil after incubation by adding 10 ml of Milli-Q water and vigorous shaking for 2 h (high-speed shaker, VWR, USA). The soil solutions were then filtered (0.45 μm; Filtropur S, Sarstedt, Germany). The

extracts were pre-concentrated by freeze-drying (Scanvac cool Safe, 100-9 Pro). After water evaporation, the sample was dissolved in 1 ml of Milli-Q water. To remove the high molecular mass compounds (>3000 Da), centrifugal ultrafiltration filters (3000 Da cutoff; Nanosep 3K Omega, Pall, Mexico) were used.

The extracted siderophores were analyzed using high-performance liquid chromatography-electrospray ionization-mass spectrometry (HPLC-ESI-MS) (Ultimate 3000 RS, Thermo Scientific, USA) including two pumps with flow rates of 0.030 and 0.15 ml/min. The pre-column (Syncronis C18, 50×2.1 mm; particle size 1.7 μm) and the separation column (Hypersil GOLD, 100×2.1 mm; particle size 1.9 μm) both were from Thermo Scientific (USA). The pre-column was eluted with mobile phase A (11 mM ammonium formate buffer, pH 4.0, and 1 % *v/v* methanol) in order to concentrate and purify the hydroxamate siderophores. After 20 min, the analytical column was loaded, and gradients of mobile phases B (11 mM ammonium formate buffer, pH 4.0, and 15 % *v/v* acetonitrile) and C (11 mM ammonium formate buffer, pH 4.0, and 5 % *v/v* acetonitrile) were applied. The ferric complexes of the hydroxamate siderophores (ferrioxamines) were detected by selected ion monitoring (SIM) of the proton adducts $[M+H]^+$, i.e., 614 for ferrioxamine B, 672 for ferrioxamine G, 656 for ferrioxamine D, and 654 for ferrioxamine E on a triple quadropole mass spectrometer (TSQ Quantum Access Max, Thermo Scientific, USA). The peaks' identities were determined using standards for each hydroxamate, in combination with using the SIM of the proton adducts that detected the known mass of each specific hydroxamate siderophore. Analyses were performed with two biological and technical replicates each to generate mean values and compute the standard deviation. Statistical outliers were calculated by use of Dean–Dixon test (Dean and Dixon 1951; Q test using critical values for confidence level of 90 %: $Q=(x_2-x_1)/(x_n-x_1)$ from data arrangement $x_1<x_2<\ldots<x_n$).

Heat stability

Heat stability of siderophores was tested by autoclaving siderophore-containing cell-free culture filtrate (SCF; for modified M9 medium see Alexander and Zuberer 1991, in HCl-washed (2 M) flasks inoculated with spores of *S. mirabilis* P16B-1; incubation at 28 °C at 100 rpm (3015, GFL shaker) for 5 days; with three biological and technical replicates). Bacterial biomass was removed by centrifugation (4000 rpm for 30 min; 4 °C; Megafuge 1.0R, Heraeus). SCF was used for hydroxamate quantification by shuttle-free CAS assay (modified from Schwyn and Neilands (1987); microtiter plates, at 630 nm in a VersaMax tunable microplate reader, Sunnyvale, CA, USA) using desferrioxamine E as a standard (Microcollections, Tübingen). In addition, SCF was analyzed via electrospray ionization mass spectrometry (Thermo Scientific LTQ XL, Germany; see Dimkpa et al. 2008a) to identify the present siderophores and detect structural alteration caused by the heat treatment.

Hydroxamate recovery

The recovery of siderophores from metal-containing soil was tested with the water-soluble fraction (pH of soil slurry was adjusted at pH 8 by use of hydroxide to allow for stable siderophore complexes; K_f at neutral pH: FOE $10^{32.5}$, FOB $10^{30.5}$; Hider and Kong 2010; Dhungana et al. 2004), shaking at room temperature for 30 min (3015, GFL shaker), incubation at 4 °C for 60 min, centrifugation at 14,000 rpm for 10 min, and filtration <45 μm of 50 g of fresh soil samples (see Schütze et al. 2013) after supplementation with SCF (iron-free solution; 81.6 μM siderophore content). The SCF itself was used as a control to calculate the initial siderophore concentration. Siderophore content was detected as described above.

Results

Identification of hydroxamate siderophores from metal-contaminated soil samples

Trihydroaxamate siderophores present in our heavy metal-contaminated soil were structurally identified by HPLC-ESI-MS as the endocyclic molecule ferrioxamine E (FOE) and the linear, acyclic molecules ferrioxamines B, D, and G (FOB, FOD, and FOG, respectively). No metal-free hydroxamates were detected, most likely due to the elevated iron and metal loads in the bioavailable fraction and total metal content in our soil (Table 1). Regarding both bioavailable metal content

Table 1 Bioavailable and total metal contents (μg/g) in multi-metal-contaminated soil after sequential extraction for mobile and specifically adsorbed fraction according to Zeien and Brümmer (1989) and total extraction

	Bioavailable metal content (μg/g)	Total metal content (μg/g)
Al	9.4±0.3[a]	64,702.1±22.4
Cd	0.7±0.003	9.6±0.4
Co	1.3±0.04	64.3±0.2
Cr	0.06±0.02	79.7±0.4
Cu	21.6±0.06	1550.6±3.8
Fe	1.6±0.02	94,056±193
Mn	61.1±3.7	990.5±2.4
Ni	7.5±0.1	324.8±3.6
U	38.2±0.7	1305.2±9
Zn	25.6±0.6	734.4±2.7

[a] n (biological)=3; n (technical)=2

(mobile and specifically adsorbed fractions) and total metal loads, different availabilities ranging from approximately 1:10 for Cd to 1:60,000 for Fe were found, with extreme contents for bioavailable Cu (21.6±0.06 μg/g), U (38.2±0.7 μg/g), Zn (25.6±0.6 μg/g), and Mn (61.1±3.7 μg/g); high loads for Al (9.4±0.3 μg/g), Ni (7.5±0.1 μg/g), and Fe (1.6±0.02 μg/g); and lower loads for Cd (0.7±0.003 μg/g), Cr (0.06±0.02 μg/g), and Co (1.3±0.04 μg/g). Cu, Ni, and Zn exceeded the maximum permissive values of the German legal guidance for the transfer of contaminants from agricultural soils into plants; Cd, Cu, Ni, and Zn total amounts exceeded the precaution values for soil in German regulations.

After the incubation period of 30 days, the total ferrioxamine concentration in the untreated control soil had decreased from 4.8 nmol/g soil to 2.7 nmol/g soil. The extracted contents of the individual molecules had changed as well (Fig. 1), with FOE and FOD decreasing while FOB increased. Values for FOG did not significantly change in non-treated soil.

In addition, after 30 days of incubation, soil mesocosms containing living biomass of either the heavy metal-resistant strain *S. mirabilis* P16B-1 or the metal-sensitive control strain *S. lividans* TK24 showed changes of siderophore contents compared with the non-treated control (Fig. 2). For the water-treated sterile control soil, high amounts of FOB (1.7 ±0.4 nmol/g soil) were detected, whereas the concentrations in inoculated microcosms were negligible (*S. mirabilis* P16B-1, 0.1±0.2 nmol/g soil; *S. lividans* TK24, 0±0 nmol/g soil). For *S. lividans* TK24, an increase of FOG was seen compared with the non-treated control and the metal-resistant strain *S. mirabilis* P16B-1.

Heat stability and recovery of hydroxamate siderophores

Heat stability was tested in SCF (cell-free culture filtrate containing siderophores produced by *S. mirabilis* P16B-1), with 93.4 % of the initial siderophore contents being detectable after heat treatment (Fig. 3). The hydroxamates were structurally identical to a non-treated FOE standard (Fig. 4).

Recovery of siderophores from soil containing SCF yielded only 32 % of the initial concentration (Fig. 5), indicating adsorption to soil particles or soil organic matter.

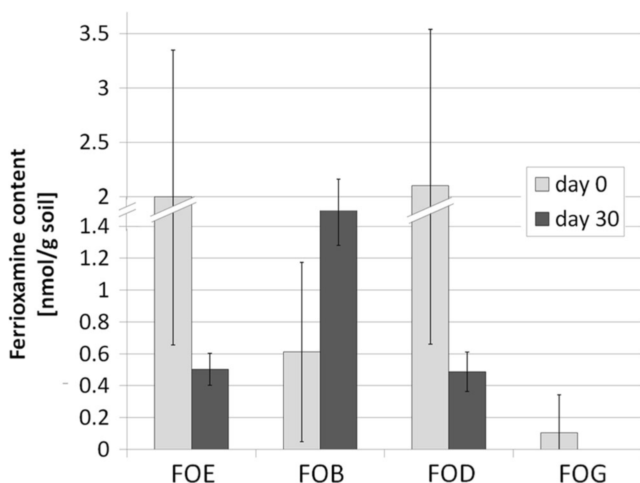

Fig. 1 Contents of ferrihydroxamates (nmol/g soil) in water-treated multi-metal-contaminated soil directly after moisturization (day 0) and after 30 days of incubation; *n* (biological)=2; *n* (technical)=2

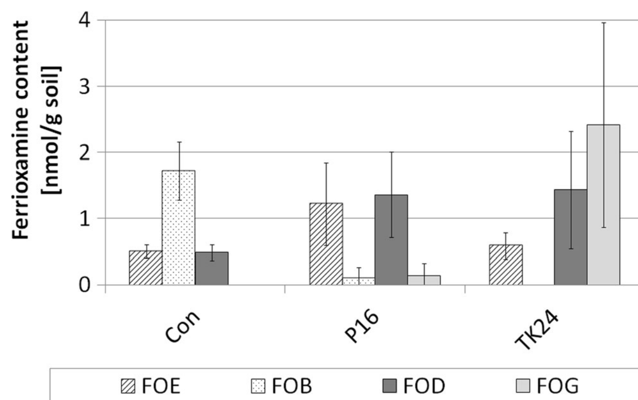

Fig. 2 Contents of ferrihydroxamates (nmol/g soil) in multi-metal-contaminated soil without (*Con*) or with the presence of heavy metal-resistant *S. mirabilis* P16B-1 (*P16*) or the sensitive control strain *S. lividans* TK24 (*TK24*) after 30 days of incubation; *n* (biological)=2; *n* (technical)=2

Discussion

The identified hydroxamates included linear ferrioxamines (FOB, FOD, and FOG) as well as the cyclic FOE (Mawji et al. 2008; Pattus and Abdallah 2000) with the cyclic siderophores being known for the higher iron–siderophore complex stability (stability constants for FOB, $K_{Fe(III)}$=30.5; FOD, $K_{Fe(III)}$=30.6; FOG, $K_{Fe(III)}$=30.8; and FOE, $K_{Fe(III)}$=32.4; Boukhalfa and Crumbliss 2002). FOB and FOE have

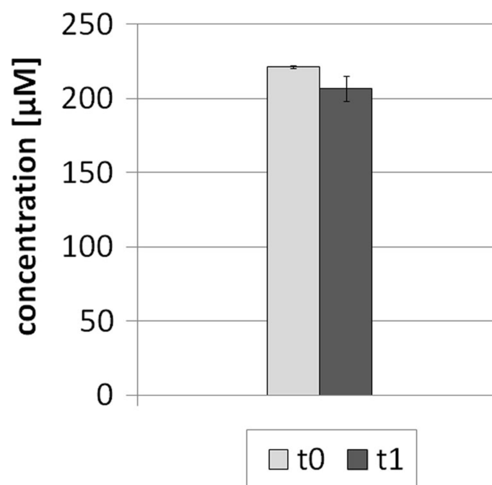

Fig. 3 Siderophore concentration (μM) of hydroxamate siderophores originating from cell-free culture filtrate (*SCF*) of *S. mirabilis* P16B-1 before (*t0*) and after heat treatment (*t1*)

Fig. 4 Chromatograms of hydroxamate siderophores from cell-free culture filtrates of *S. mirabilis* P16B-1 (**a**, **b**) compared with a DFOE (*red*) and FOE (*green*) standard (**c**, **d**). Measurements were performed before (**a**, **c**) and after heat treatment (**b**, **d**). DFOE was detected at 601 *m/z* and FOE at 654 *m/z*

different charge properties, and FOD is the uncharged acetyl derivative of FOB (Cocozza et al. 2002; Haack et al. 2008). Thus, with incubation of the water-treated soil, an alteration between neutral and cationic forms and (de)acetylation was observed. Regarding the decrease of the cyclic FOE in non-inoculated soil, a linearization of hydroxamates in soil can be assumed. Our results thus provide, for the first time, evidence for an abiotic structural alteration of hydroxamate siderophores in soil. Both auto-alteration and enzymatic action in soil potentially may provide the observed functionalities.

The linear ferrioxamines, indeed, have been shown to chelate alternative metal ions (Dimkpa et al. 2008b, 2009a; Rajkumar et al. 2010), thus potentially alleviating metal stress to soil (micro)biota. This, of course, is dependent on the recognition of iron versus alternative-metal-bound siderophore molecules prior to uptake. To allow the cells to distinguish between Fe and non-Fe metal–siderophore

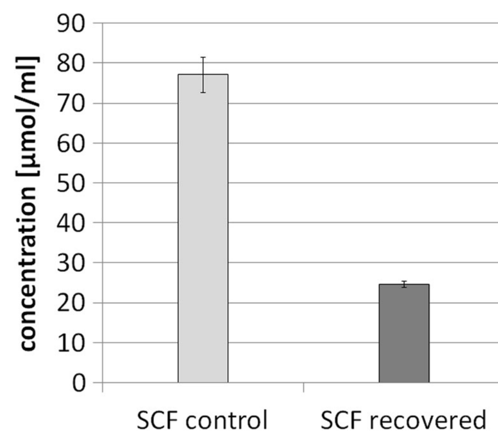

Fig. 5 Hydroxamate siderophore recovery from multi-metal-contaminated soil: Concentration of siderophores present in cell-free culture filtrate (*SCF*) of *S. mirabilis* P16B-1 (*control*) and after recovery from heavy metal-contaminated soil by water extraction (*recovered*)

complexes, a high metal specificity of the ferri-siderophore pathway is needed. For pyochelin systems requiring the uptake of siderophore–metal complexes into the cytoplasm, it is known that only Fe could activate its biosynthesis. Such a mechanism prevents bacterial cells from intracellular metal toxicity (Saha et al. 2013). Nevertheless, the introduction of non-Fe metals into the cell via pyochelin is reported to be likely (Schalk et al. 2011). Thus, further studies are needed to elucidate the distinct role of siderophores and their specific uptake receptors for delivery of metals into bacterial cells.

The presence of Al, Cd, Ni, and Cu induced siderophore production by heavy metal-resistant streptomycetes (Dimkpa et al. 2008b). FOE and FOB produced by the multi-metal-resistant *Streptomyces acidiscabies* E13 chelated high amounts of Ni and Fe, respectively. The authors reported that the amount of bound Fe and Ni, respectively, was dependent on siderophore type and time. Hydroxamate analysis took place under Fe deficiency caused by high Ni levels at four time points reflecting different steps of bacterial growth. FOE was found to bind more Ni (especially at 72 and 96 h), whereas FOB showed a higher preference for Fe chelation (24 h Fe>Ni; 48 h Fe≈Ni; 72 h Fe>Ni; 96 h Fe<Ni). In contrast, catecholate siderophores were shown to bind relatively low amounts of metals (coelochelin, even at the time point where maximum levels had been seen, remained metal-free; Dimkpa et al. 2008a). Thus, siderophores may provide different functions in soil, with ferrihydroxamate types possessing different metal binding preferences. Dimkpa et al. (2009b) conducted siderophore competition assays between Al and Fe, and Fe and Cu showing (LC–ESI-MS) 27 % of Al bound to FOE being displaced by Fe, whereas 18 % of bound Fe was replaced by Al. In contrast, Cu showed low affinity to FOE with 80 % of FOE being Cu-free. The low amount of bound Cu was completely displaced by Fe, whereas the presence of Cu did not cause replacement of bound Fe. These data strongly suggest that trivalent metals are more competitive for siderophore binding than divalent ones. For FOB, different metal formation constants were calculated including the parameter metal ion charge ratio, ionic radius, metal–ligand interatomic separation, and metal ion first hydrolysis constant which were used to predict affinity. The method was validated by comparison of predicted data with measured metal concentrations and further applied for untested metals. The order of complex stabilities for FOB is given as CaII<NiII<ZnII<CuII<AlIII<FeIII (Hernlem et al. 1999).

Since our metal-contaminated soil showed elevated concentrations for Zn, Al, Ni, Cu, and Fe, it can be assumed that other non-Fe metals are competing with Fe for siderophore binding. This was pronounced in experiments containing the living biomass of the metal-resistant *S. mirabilis* P16B-1, where a higher impact on metal immobilization was seen as compared with the sensitive control *S. lividans* TK24 (*S. mirabilis* P16B-1: 5 metals in F1, 17 metals in F2 including 11 heavy metals and 9 REE; *S. lividans* TK24: 6 metals in F1 and 5 metals in F2 including 9 heavy metals and 1 REE). The increases in F1 and F2 fraction metal contents compared with the water-treated control clearly were lower, where the increase of bioavailable metal loads over time in the control most likely was due to AMD formation (Schütze et al. 2014). In our extremely metal-contaminated soil, the control strain *S. lividans* TK24 was shown to starve and die, whereas the metal-resistant strain was shown to survive and grow which seems to be causal for their different metal alteration pattern. Morphological investigation via scanning electron microscopy revealed either healthy and vital mycelium with occasional hyphal tip formation for the metal-resistant strain or empty cell envelopes and disintegrated cell structures for the sensitive strain after 30 days of incubation. No re-isolation was possible for the sensitive *S. lividans* TK24. In addition, strain-specific DNA contents were measured by qPCR which could show constant DNA amounts for the metal-resistant *S. mirabilis* P16B-1, whereas DNA of the sensitive strain strongly declined (Schütze et al. 2014).

Thus, two independent processes seem to cause the detected decrease in FOB. In the case of the sensitive strain, a decrease of FOB with subsequent increase of FOG was observed. This strongly indicates auto-alteration of hydroxamates. In contrast, incubation with the surviving, metal-resistant *S. mirabilis* P16B-1 leads to FOB decrease without increase of any other ferrihydroxamate in the given extent. Thus, it seems likely that FOB was taken up and potentially metabolized by the bacteria. To the best of our knowledge, the consumption of FOB has been reported only for heterotrophic nitrifiers using FOB as carbon source (Castignetti and Siddiqui 1990). In case of the heavy metal-resistant streptomycete, FOB likely was used to provide energy or building blocks for other metabolic processes. Only a low increase of FOD was seen, which might be related to siderophore production by this strain to overcome iron deficiency in soil.

Heat stability of commercial ferrioxamine B has been reported (Desferal by Ciba-Geigy, Switzerland; see Bossier and Verstraete 1986). In addition, ferrioxamine hydroxamates were demonstrated to be stable in seawater and do not degrade photochemically (Mawji et al. 2008). Thus, their structural stability in the presence of salts, heat, and natural sunlight indicates for other mechanisms inducing auto-alteration in metal-contaminated soil. From plant experiments (Dimkpa et al. 2009b) and clinical use (Adgent et al. 2012), an interaction with and inhibition of free radicals is known. Fe and non-Fe metal-mediated Fenton reactions (Nagajyoti et al. 2010; Shestivska et al. 2011) in heavy metal-contaminated soils are known to be responsible for high amounts of reactive oxygen species (ROS) which may cause structural alteration of molecules present in soil. As the redox conditions of sediments and soils have important impact on metal transition (Guo et al. 1997), there might also be an effect on siderophores. In

addition, aggressive pH due to AMD may be involved in autoalteration of hydroxamates. Our metal-contaminated soil showed a pH of 5.72 (Schmidt et al. 2008) which makes an impact of ROS on abiotic hydroxamate alteration likely. The mechanisms involved in the observed alteration await more detailed analyses.

Metal availability in the mobile and easily mobilized fractions in our metal-contaminated soil strongly decreased in the presence of living biomass of the extremely metal-resistant *S. mirabilis* P16B-1 (Schütze et al. 2014). Thus, there seems to be a relation between resistance mechanisms depending on living cells, including siderophore production, and metal immobilization. The high amount of non-extractable siderophores indicates for a considerable impact of soil-bound non-Fe metal–siderophore complexes to metal alteration in soil. As (former) mining areas usually feature multi-element contaminations (Langella et al. 2014), investigation of chelation prevalences to non-Fe metals will help to understand resistance mechanisms of heavy metal-tolerant bacteria. Thus, a deeper understanding of non-Fe metal chelation by ferrihydroxamates, including the impact of metal bioavailability, charge and hydrodynamic radius of metals, and competition and stability of complexants, may help to understand the processes important specifically with environmental issues of metal contamination in Western Europe. Here, the application of metal-resistant soil bacteria (Phieler et al. 2013) producing siderophores in the presence of high metal loads would open up new roads for bioremediation approaches.

In summary, the low recovery of hydroxamates from soil may be beneficial for soil-inhabiting (micro)organisms by metal immobilization. To the best of our knowledge, consumption of siderophores was shown for streptomycetes for the first time, most likely to overcome nutrient limitation in the extremely poor, metal-contaminated soil.

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