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Arousing sleeping genes: shifts in secondary metabolism of metal tolerant actinobacteria under conditions of heavy metal stress

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Abstract Numerous microbial habitats are strongly influenced by elevated levels of heavy metals. This type of habitat has developed either due to ore mining and metal processing or by pedogenesis above metal-rich base rocks. Most actinobacteria are soil-borne microbes with a remarkable capability for the synthesis of a broad variety of biologically active secondary metabolites. One major obstacle in identifying secondary metabolites, however, is the known phenomenon of sleeping gene clusters which are present, but silent under standard screening conditions. Here, we proceed to show that sleeping gene clusters can be awakened by the induction in heavy metal stress. Both, a chemical and a biological screening with extracts of supernatant and biomass of 10 strains derived from metal contaminated and non-contaminated environments was carried out to assay the influence of heavy metals on secondary metabolite patterns of metal tolerant actinobacteria. Metabolite patterns of cultures grown in complex and minimal media were compared to nickel (or cadmium) spiked parallels. Extracts of some strains grown in the presence of a metal salt displayed intense antibiosis against *Escherichia coli*, *Mycobacterium smegmatis*, *Staphylococcus aureus* and *Candida albicans*. Contrarily to the widely held opinion of metals as hindrance in secondary metabolism, metals thus can induce or enhance synthesis of possibly potent and medically relevant metabolites in metal tolerant strains. Hence, re-screening of existing strain libraries as well as identification of new strains from contaminated areas are valid strategies for the detection of new antibiotics in the future.

Keywords *Actinobacteria* · Antibiosis · Heavy metal · Screening program · Secondary metabolism

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Introduction

During a particular stage in the life cycle synthesis of secondary metabolites, among them numerous antibiotics, occurs in various prokaryotes, e.g., *Actinobacteria*, *Myxobacteria* and *Bacillus*. Several eukaryotes like fungi, and a great number of plants also possess the capabilities of potent secondary metabolism. *Actinobacteria* contribute with two-thirds of the total the lion's share of the antibiotics producers. The total number of antibiotics is stated, depending on the author, with 5,000 (Demain and Fang 2000), or more than four times this number (Berdy 2005). The objective of screening programs for detection of bioactive compounds has switched

from antibiosis assays performed mostly up to the 1990s to other types of assays that led to new discoveries, without which "there would be a significant therapeutic deficit in several important clinical areas, such as, neurodegenerative disease, cardiovascular disease, most solid tumors, and immune-inflammatory disease" (Nisbet and Moore 1997). Nevertheless, antibiotics still remain the largest market of naturally derived drugs with 67% sales in 2000 (Bull et al. 2000), even if the discoveries of microbial metabolites with non-antibiotic activities now exceed that of antibiotic compounds (Hill et al. 1998). The identification of new antimicrobial substances is a major aim in medical microbiology since resistance to known antibiotics makes the identification of new substances mandatory.

To get access to new microbial metabolites, several strategies can be applied, e.g., development of selective isolation procedures for rare actinomycetes and less thoroughly analyzed *Streptomyces* clusters (Sanglier et al. 1993) that are considered as promising producers. Analysis of 16S rDNA in soil samples by help of taxon specific primers is used to predict the presence of desired groups before starting specific isolation procedures (Donadio et al. 2002). A comprehensive review on search strategies is given by Bull et al. (2000). Additionally, optimization of the fermentation medium's composition to increase the yield of secondary metabolites is in the focus of various studies (reviewed by Iwai and Omura 1982).

However, one major concern is that production of potential secondary metabolites is under regulation and hence many putative drugs are overlooked in screening programs due to the fact that they are not induced under the tested conditions. Such gene clusters have been named sleeping gene clusters (Hopwood 2006). The influence of metals in the fermentation medium as inductors or enhancers of secondary metabolism has not yet been studied systematically. To gain insight into the effects that heavy metals can have on secondary metabolite production, we tested 10 selected, metal tolerant actinobacteria strains from contaminated, uncontaminated and naturally metal-enriched environments. The aim of this investigation was to compare secondary metabolite patterns and antibiosis activities of metal spiked with non-spiked cultures. Due to the observation that secondary metabolism can be linked to the stress response of the organism as can be seen in, e.g., melanogenesis, it was expected to find changed patterns of secondary metabolites in metal treated cultures.

Materials and methods

Soil sample collection and isolation of actinobacteria

Three soil samples were collected at a former uranium mining site in Eastern Thuringia, Germany (foot and top at the re-vegetated waste heap "Stolzenberg" and periphery of the seepage water reservoir "Pohlteich" characterized by deposits of secondary minerals). One sample originated from the serpentinite rich soil of Pieve Santo Stefano, Tuscany, Italy. Two non metal-enriched soil samples were taken from a public garden (Paradies) and the dry grassland of Windknollen (Napoleonstein) of Jena, Thuringia, Germany. The soils were air dried, ground and resuspended in 0.9% NaCl-solution and agitated for 1 h. 100 µl aliquots of 10^{-3}–10^{-5} dilutions were plated on soil extract agar (Thiemann and Beretta 1968). After 5 days of incubation at 28°C, pure cultures were obtained on minimal medium for actinobacteria (0.5 g l^{-1} asparagine, 0.5 g l^{-1} K_2HPO_4, 0.2 g l^{-1} $MgSO_4$, 0.01 g l^{-1} $FeSO_4$, 10 g l^{-1} glucose, 15 g l^{-1} agar; Amoroso et al. 2000). All isolates are deposited in the strain collection at the Leibniz Institute for Natural Compound Research and Infection Biology (HKI, Jena).

Phenotypic characterization and taxonomy

In order to obtain taxonomic information, the selected strains were classified at least on the genus level. Genomic DNA was isolated following the CTAB method (Kieser et al. 2000) and used for PCR with 16S rDNA specific primers (TPU1 AGAGTTTGATC MTGGCTCAG and RTU3 GWATTACCGCGGCK GCTG, Choi et al. 1994). The amplified 500 bp fragments were cloned and sequenced (JenaGen, Jena, Germany). Blast analyses were performed to identify similarities to database entries (NCBI).

Test for metal tolerance

Strains were streaked on plates containing metal supplemented minimal medium. Growth was observed

and estimated visually after 7 days of incubation at 28°C and compared with growth on non-supplemented minimal medium. The metals $NiCl_2 \cdot 6H_2O$ and $CdCl_2 \cdot 6H_2O$ were added as 0.2 μm sterile filtered solution after autoclavation and before solidification of the medium. The final concentration of each metal in the plate was 1, 5 and 10 mM.

Cultivation and preparation of crude extract

The strains were grown in plate culture on starch casein medium (10 g l^{-1} soluble starch, 1 g l^{-1} casamino acids, 0.5 g l^{-1} K_2HPO_4, 16 g l^{-1} agar) until sporulation (7 days at 28°C). Spores were harvested with 5 ml sterile 0.9% (w/v) NaCl solution per plate. One millilitre spore suspension was used as inoculum for a first preculture, consisting of 10 ml minimal medium in 50 ml Erlenmeyer flasks. Cultures were incubated at 28°C for 2–4 days on a rotary shaker. These cultures were used as inoculum for the subsequent preculture in 500 ml Erlenmeyer flasks containing 100 ml minimal medium. Cultures were incubated for 48 h at 28°C. Ten millilitre of the second preculture were used as inoculum for the main culture, which was either soy-mannite medium (20 g l^{-1} soy meal and 20 g l^{-1} mannite, pH adjusted to 7.2) or minimal medium. Five hundred millilitre Erlenmeyer flasks were used for 100 ml culture volume. For induction of stress response, heavy metals were added to give a final concentration of 0.3 mM $NiCl_2 \cdot 6H_2O$ or 45 μM $CdCl_2 \cdot 6H_2O$ (only for cadmium resistant strain F4).

After 5 days of incubation the culture broth was separated by centrifugation into supernatant and mycelial fraction in order to test cytosolic and excreted compounds independently. For the supernatant, solid phase extraction (Amberchrom 161c) was applied. Extracting solvent was methanol. Likewise, the mycelial fraction was extracted with methanol.

Chemical screening by thin-layer chromatography (TLC)

Extracts of supernatant and mycelia were analyzed by thin-layer chromatography. Two different running systems were applied; butanol:glacial acetic acid:-water = 4:1:5 and chloroform:methanol = 9:1. For detection of chemical compounds of different substance groups, thin layer chromatograms were developed using UV light at wave length 254 and 366 nm as well as staining with anis aldehyde, Ehrlichs reagent and orcinol.

Bioassay

Extracts of supernatant and mycelia were tested for antibacterial and antifungal effects in an agar diffusion assay. *Staphylococcus aureus*/MRSA + chinolon-r 134/93, *Escherichia coli* SG 458, *Mycobacterium smegmatis* SG 987 and *Candida albicans* BMSY 212 were used as test organisms. Fifty microlitre of the single extracts were filled in 9 mm agar wells. The antibiotic effect was evaluated by measuring the zones of inhibition.

Results and discussion

Isolation of heavy metal tolerant actinobacteria

Three different soil types were used to identify actinobacteria with presumably different levels of heavy metal tolerance. While non-contaminated soils should be expected to yield only strains which by chance were deposited there, higher levels of resistance should be expected to be found in soils evolved on naturally metal-rich ultramafic rock. Samples from covered mining heaps and the samples from highly contaminated acid mine drainage sites might be expected to show multiple and high-level resistance towards heavy metals. We tested heavy metal tolerance towards two heavy metals, nickel and cadmium. The ten isolates with the most pronounced tolerance were chosen from 100 isolates derived from the three environments. Five of the selected strains originated from the former uranium mining area, three from the ultramafic biotope on serpentine soil and two from uncontaminated habitats.

All strains displayed clear tolerance towards nickel, whereas only strain F4 was able to grow in presence of 1.0 mM cadmium. About 1.0 mM nickel was tolerated by four strains from the mining area, two strains from ultramafic soil and one strain from an uncontaminated habitat. 5 mM was tolerated by two strains, one from the mining area and one from uncontaminated soil. The strain Tosca3, originating from the ultramafic soil in Tuscany, was capable of growing in presence of 10 mM nickel, which can be

considered as a marked resistance. For the 10 selected isolates, taxonomic determination was performed by 16S rDNA sequencing (Table 1).

Comparison of products from crude extract of supernatant versus mycelium

After 7 days of shaking flask culture at 28°C, supernatant and biomass were separated by centrifugation and subsequently extracted. The extracts were used in a chemical and a biological screening. The chemical screening is used as a cost-effective, first screening procedure. To detect different chemical compounds, thin-layer chromatograms were developed using UV light as well as staining techniques. Putative bioactive compounds were found both in the mycelium (e.g., strain F4) or released into the culture broth (e.g., strain PT1). Therefore, both fractions were used for further screening procedures.

Natural compounds produced on rich versus minimal medium

When strains are cultured solely in the usual fermentation media, compounds derived from sleeping gene clusters might be overlooked. Strains grown in minimal medium showed indeed a clearly reduced number of extractable metabolites, both in the supernatant and in the mycelium. However, some of the crude extracts contained metabolites which seemed to be synthesized solely in minimal medium. Examples can be seen in anisaldehyde-detected TL chromatograms of strains JE12, E13 and Tosca4 (Fig. 1). Hence, we propose to use a minimal medium in addition to rich fermentation media for evaluation of secondary metabolite production.

Heavy metal induction of secondary metabolites

By chemical screening heavy metals were found to induce production of secondary metabolites in complex as well as minimal media. Most of the metabolites are produced in both the metal-free and metal-containing media. Many bands of metabolites in TLC can be found only in metal-free cultures. However, bands of metabolites can be found that appear only in metal spiked cultures, e.g. in extracts of mycelium of strain Tosca3 grown in minimal medium with $NiCl_2$ (Fig. 2). Likewise, growth of strain Tosca3 in soy-mannite medium supplemented with nickel displayed additional substance bands. Mycelium of strain JE12 displayed a band in complex medium induced by metal supplementation (Fig. 3). Strain PT1 in turn showed a band in the supernatant of a minimal medium grown culture, if metal was supplemented (Fig. 3). Strain PT13 showed a prominent band in the extract of the supernatant after growth in complex medium (Fig. 3). The results indicate that for metal spiked cultures, no trend is to be observed: the unique metabolites are produced either only in minimal or in complex media with or without metal addition. In addition, unique metabolites are excreted into the medium, or can be found in the mycelium.

Table 1 Taxonomy of metal tolerant strains and resistance concentrations

Strain	Sampling site/date	Taxon	Resistance
E13	Waste dump Stolzenberg, 1999	*Streptomyces acidiscabies*	5 mM Ni
F4	Waste dump Stolzenberg, 1999	*Streptomyces tendae*[a]	1 mM Cd/1 mM Ni
PT1	Seepage pond, Pohlteich, 2003	*Streptomyces ciscaucasicus*	1 mM Ni
PT5	Seepage pond, Pohlteich, 2003	*Streptomyces* sp.	1 mM Ni
PT13	Seepage pond, Pohlteich, 2003	*Streptomyces aureus*	1 mM Ni
Tosca2	Pieve San Stefano, 2002	*Streptomyces purpurascens*	1 mM Ni
Tosca3	Pieve San Stefano, 2002	*Streptomyces lincolnensis*	10 mM Ni
Tosca4	Pieve San Stefano, 2002	*Lentzea waywayandensis*[b]	1 mM Ni
JE12	Napoleonstein, grassland, 2002	*Kitasatospora* sp.	5 mM Ni
WiP14	Paradies, public garden, 2003	*Streptomyces* sp.	1 mM Ni

[a] *S. tendae* formerly described as *S. rochei*

[b] *L. waywayandensis* as *Saccharothrix waywayandensis*

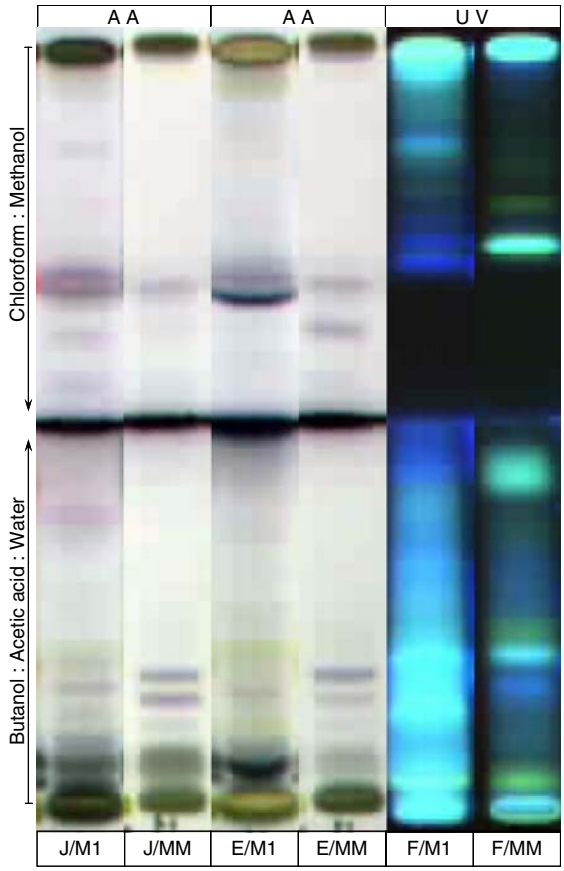

Fig. 1 Extracts of strains JE12 (J)—mycelial fraction, E13 (E)—mycelial fraction and F4 (F)—supernatant on TLC. Growth without metal addition in soy-mannite medium (M1) and minimal medium (MM) shows media dependency of metabolite production. Detection: anis aldehyde (AA) and UV at 366 nm (UV)

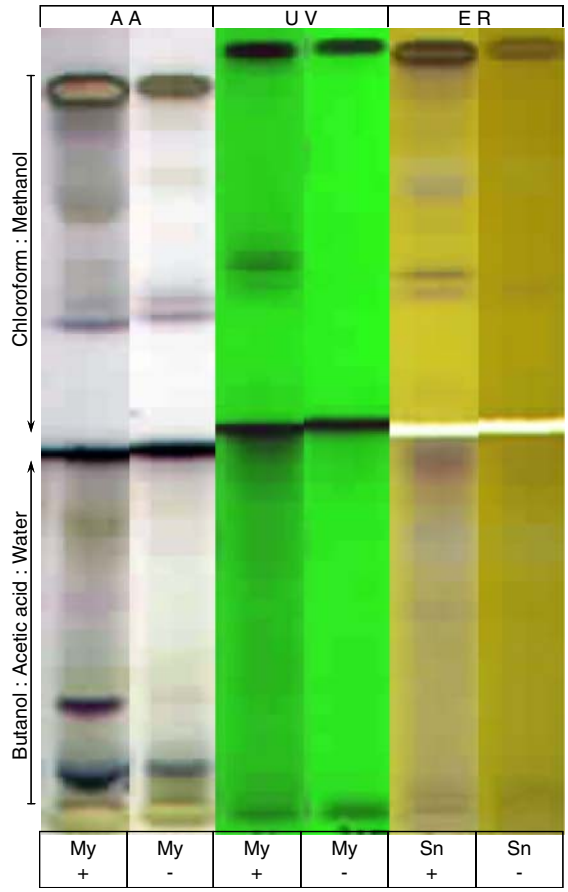

Fig. 2 Extracts of the mycelial (My) and supernatant (Sn) fraction of strain Tosca3 grown in minimal medium (MM) with (+) or without (−) nickel supplementation on TLC showing metal induced metabolites. Detection: anis aldehyde (AA), UV at 245 nm (UV) and Ehrlich's reagent (ER)

Antibiotic compounds

The activity of metabolites produced at low concentrations can be detected in a biological screening (Table 2). Some of the crude extracts from metal spiked cultures showed biological activity towards several medically relevant test organisms. Strains JE12 and E13 displayed activity only with extracts of the metal grown cultures. This suggests a molecular mechanism of induction due to metal supply. It remains to be tested, whether the compounds produced in these cultures are synthesized only in presence of metals, or if their production is induced under metal-rich conditions to amounts exerting biological activity. In the bioassay, the mycelial fraction of strain JE12, an isolate of a non-contaminated soil, showed a strong antibiosis towards test organism *M. smegmatis* only after growth in nickel-supplemented soy-mannite medium, and a moderate antibiosis against *S. aureus* only after growth in nickel-supplemented minimal medium. There was no antibiosis detected in the non-spiked media for strain JE12.

The mycelial fraction of strain PT1 as isolate of a mining area displayed a potent antibiosis towards *C. albicans* only after growth in nickel-supplemented soy-mannite medium. The mycelial fraction of another isolate from the mining area, strain E13, displayed antibiosis towards *S. aureus* and *M. smegmatis* after growth in nickel treated soy-mannite medium. Two of the three isolates from an ultramafic soil showed effective antibiosis after

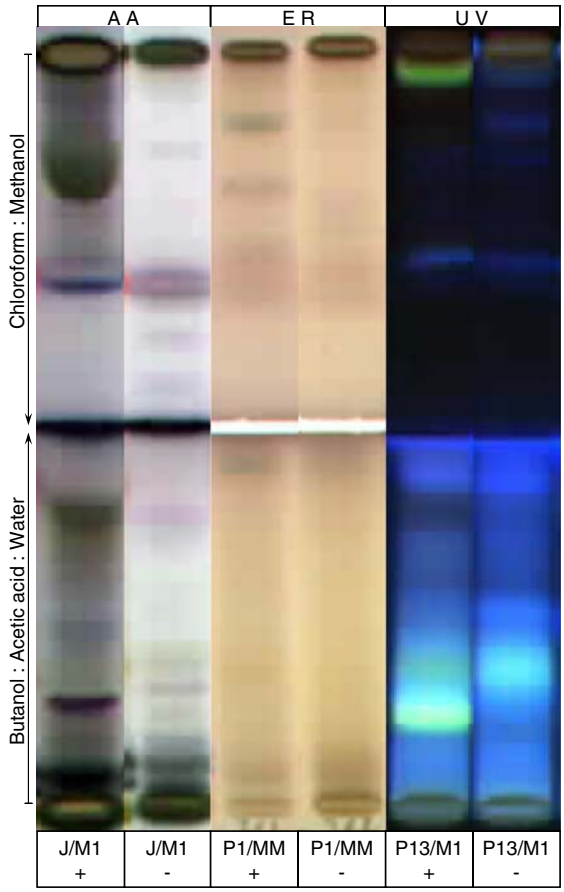

Fig. 3 Extracts of strains JE12 (J)—mycelial fraction, PT1 (P1)—supernatant and PT13 (P13)—mycelial fraction on TLC showing metal induced metabolites. Strains grown in either soy-mannite (M1) or minimal medium (MM) with (+) or without (−) nickel supplementation. Detection: anis aldehyde (AA), Ehrlich's reagent (ER) and UV at 366 nm (UV)

growth in the presence of nickel. The supernatant fraction of strain Tosca2 was able to inhibit *E. coli*, whereas the mycelial fraction of strain Tosca3 was effective towards *S. aureus*. Hence, screening procedures should be re-evaluated for heavy metal induction of strains isolated from non-contaminated biotopes (like strain JE12), and isolation of strains is proposed to include specifically heavy metal-rich environments to yield heavy metal tolerant actinobacteria like strains E13, Tosca2 or Tosca3.

Metabolite–metal interactions

Many soil habitats in mining regions are enriched in heavy metals. What is the influence that excessive metal concentration in soil has on secondary metabolism? It can be hypothesized that synthesis of metabolites which detoxify heavy metals by chelation is increased when metals are added to the fermentation medium of heavy metal tolerant strains. It has been shown that many antibiotics and other secondary metabolites, for example isatin, a metabolite of *S. albus*, can scavenge heavy metals from the medium (Gräfe and Radics 1986). The polyketide gamma-actinorhodin produced by *S. coelicolor* has been described as a chelator of iron (Coisne et al. 1999), and the secondary metabolite melanin has been shown to be involved in heavy metal tolerance in *S. scabies* (Beausejour and Beaulieu 2004).

Raising the concentration of trace metals in fermentation media from usually 10^{-7} M by 10–100-fold is required for metabolite production (Iwai and Omura 1982). The concentration of nickel that has been applied in the presented assay is with 3×10^{-4} M above the "threshold of production". Such high concentration requires tolerance as it is considered inhibiting growth of sensitive strains. If, under the conditions of heavy metal stress, growth still occurs and metabolite production proceeds, a stress response has been initiated, which usually is linked to a switch in metabolism. Both, the supply of nickel to Ni^{2+} dependent enzymatic steps and the cytosolic or extracellular chelation of nickel by metabolites is thought to play a key role in production of a specific set of secondary metabolites in nickel-spiked media.

Microorganisms adapted to life in hazardous environments are considered weak producers of antibiotics, reasoned to be due to the lack of competition within the microbial community of the habitat (Vining 1990). With this study we could show that metal tolerant isolates of the group of actinobacteria, originating from metal-enriched habitats of a former uranium mining site are capable of strong antibiosis towards various test organisms, even if extracted from soil and sediment samples of low cfu values (Schmidt et al. 2005). Likewise, Sprocati et al. (2006) showed the influence of heavy metals on the metabolic profile of metal resistant, yet unidentified filamentous and single-celled bacteria from an abondened mine. They showed that in the presence of a heavy metal, some of the 74 investigated substrates are utilized with higher oxidation rates (Sprocati et al. 2006). A metabolic shift due to growth in nickel or cadmium spiked cultures consequently leads to

Table 2 Biological screening of extracts of supernatant (S) and mycelial fraction (M)

Isolate	Culture +/− supplement	*S. aureus* 134/94		*E. coli* 458		*M. smegmatis* 987		*C. albicans*	
		S	M	S	M	S	M	S	M
F4 (ums)	M1 + Cd		3				3		
	M1		3				3		
	MM + Cd								
	MM								
E13 (ums)	M1 + Ni		2				3		
	M1								
	MM + Ni								
	MM								
PTI (ums)	M1 + Ni		2				3		3
	M1		2				3		
	MM + Ni								
	MM								
PT5 (ums)	M1 + Ni	3						3	
	M1	3					1	2	
	MM + Ni								
	MM	2	2	3			1		
PT13 (ums)	M1 + Ni						3		
	M1						3	2	
	MM + Ni								
	MM						2		
Tosca2 (ser)	M1 + Ni	3	3	2		3	3		
	M1	3	3			3	3		
	MM + Ni	3	3			2	2		
	MM	3	3			3	3		
Tosca3 (ser)	M1 + Ni	3	3				3		
	M1	3					3		
	MM + Ni		3						
	MM								
Tosca4 (ser)	M1 + Ni						3	3	
	M1						3	3	
	MM + Ni								
	MM								
JE12 (nme)	M1 + Ni						3		
	M1								
	MM + Ni		2						
	MM								
Wip14 (nme)	M1 + Ni	3		1		2		2	
	M1	3				3		2	
	MM + Ni						2		
	MM	3				2	2		

Blank, no inhibition; 1, moderate inhibition; 2, inhibition; 3, strong inhibition; M1, soy-mannite medium; MM, minimal medium; isolates originating from habitats of an uranium mining site (ums), serpentine soils (ser) and non-metal-enriched locations (nme)

formation of different secondary metabolites or metabolites in altered concentration, in our study.

Metal resistance and bioactivity of strains from contaminated versus uncontaminated habitats

A nickel adapted and highly nickel tolerant microflora has evolved in habitats that developed due to pedogenesis above ultramafic parent rocks, as shown by Mengoni et al. (2001) for an area in Tuscany, Italy, and for neocaledonian soils (Hery et al. 2003). This type of pedogenesis results in 1.2–2.0 mg nickel per gram soil dry weight in nickel-rich serpentine soils (Mengoni et al. 2001). Here, we isolated the strain Tosca3, displaying the most pronounced resistance (for calculating concentration of resistance see Duxbury 1981; Trevors et al. 1985), from the ultramafic soil sample of Tuscany. There are only very few studies on antimicrobial characteristics of isolates originating from this type of soil. A strain of the new species *Streptomyces yatensis* isolated from a New-Caledonian ultramafic soil revealed a remarkable spectrum of antimicrobial and antitumor activities (Saintpierre et al. 2003).

However, tolerance can be observed even in strains originating from uncontaminated soils, as shown here. A shift in secondary metabolites under the influence of metals has been observed with each type of isolate, originating from long adaptation on ultramafic soil (Tosca2, Tosca3), short-term adaptation at mine sites (E13), or non-contaminated areas (JE12).

In contrast to our expectation to find the highest metal resistance among isolates of the mining area, strain Tosca3, originating from serpentine soil of Tuscany, displays the most distinct resistance towards nickel. An explanation can be given by help of the concept of "ecological islands" by Kruckeberg (1984) and Lefèbvre and Vernet (1990). The mineral composition of the serpentine soil with its extremely high concentrations of certain heavy metals like nickel and the time span for evolution of organisms dwelling in that habitat may lead to distinct adaptation.

The former uranium mining site was 40 years in operation. Thus, the time given for adaptive responses was comparatively limited. Resistance of microbes in close contact with metal containing mining waste was less developed as compared to those in soil that was formed by pedogenesis above ultramafic parental rock for millions of years in this naturally occuring type of habitat.

Stress induction and media design

The genomes of *Streptomyces avermitilis* and *Streptomyces coelicolor* are predicted to encode at least 25 (Omura et al. 2001) and 22 (Bentley et al. 2002) different secondary metabolites, respectively. It is not clear under which culture conditions a certain pattern of secondary metabolites is produced. Supplementation of minimal or complex media with heavy metals as simulation of environmental conditions to which the strain might have adapted, can induce synthesis of hitherto undiscovered metabolites.

Synthesis of secondary metabolites of strains grown in minimal medium has to be compared with soy-mannite medium. Soy-mannite medium resembles in a high degree a typical fermentation medium for optimal product yield and can be considered as a (semi)rich medium. Minimal medium, however, reflects far better the nutritional situation in most natural habitats. Secondary metabolites produced under natural conditions, effective in microbial soil habitats should be screened using a medium that comes close to the conditions occurring in the habitat in terms of, e.g., carbon supply, pH or concentration of trace elements. It has been shown that isolates from an anthropogenically heavy metal contaminated habitat (E13), a naturally nickel-enriched soil (Tosca4) and a non-disturbed grassland soil (JE12) produce metabolite(s) in minimal medium that do not appear in the extracts of soy-mannite grown cells. This suggests that the capability of secondary metabolite production is not exhausted by testing the common fermentation media. Rather, minimal media can induce formation of secondary metabolites which otherwise would remain undiscovered. From an ecological perspective, secondary metabolites produced in minimal media are very likely the ones that play a dominant role in the soil habitat at the transition phase of the producer organism, when vegetative mycelium breaks down and "fatal attraction" starts to occur. In an extension, it might be speculated that a screening for characteristic secondary metabolite patterns should include shaking flask as well as solid fermentation in soil extract media as even more natural substrates.

The manifold and often opposed effects of different heavy metals on secondary metabolism have been studied in detail with *S. galbus*. Cadmium and chromium stimulated pigment production at lower concentrations, whereas nickel and mercury negatively affected the production. In contrast to this finding, it was reported that nickel has a stimulatory effect on *S. rishiriensis* in producing the antibiotic coumermycin A1 (Claridge et al. 1966). The production of actinorhodin of *S. coelicolor* has been shown to be sensitive to mercury, cadmium, copper, nickel and lead, but can be slightly stimulated by chromium. From these aspects and based on the presented results of 10 strains of actinobacteria, we conclude that patterns of secondary metabolites are strongly changed upon addition of different heavy metals. The pattern depends on both, the kind of the metal added and the metal concentration.

The concentration of 45 μM Cd for stress induction of strain F4 is considered high. For this element, no biological function has been shown. In contrast, the uptake of the element nickel with its essential function in a number of enzymes, like hydrogenase, urease and superoxide dismutase, has to be homoeostatically regulated. Therefore, the concentration for stress induction needs to be higher and has been chosen with 0.3 mM in the present study.

Isolates from both environments, non-contaminated and contaminated, can display similar responses, as could be shown for the biological activity of strains E13 (isolate from contaminated site) and JE12 (isolate from non-contaminated site). Interestingly, the two strains show biological activity only after growth in presence of nickel. The effect on secondary metabolism induced by metals is thought to be part of a stress response and can be exploited for the search of novel metabolites. It remains to be tested, whether this activity is nickel specific, or can be induced also by other metals to which the strains show tolerance. The strong biological activity of strain PT1 towards *C. albicans* after growth on nickel supplemented medium seems to warrant further studies.

Conclusion

It has been demonstrated by the two independent procedures of biological and chemical screening that the secondary metabolite patterns of selected strains can vary under the influence of heavy metals added to the fermentation medium. Some metabolites are produced in raised concentration; others are produced solely in the presence of metal. Strains isolated from contaminated, as well as from non-contaminated habitats display an altered secondary metabolite pattern visualized by TLC and varied antibiotic activity in bioassays after growth in metal containing medium. This suggests that not only actinobacterial strains that are adapted to life with high metal concentration can be influenced in their secondary metabolism by metals, but isolates originating from undisturbed environments show similar responses. It certainly would be worthwhile to re-screen already established microbial strain collections for novel metabolites produced under the influence of heavy metals. The background of the intensified synthesis of particular secondary metabolites very likely is the interplay of the metal dependency of many enzymatic synthesis steps, a metal chelating effect due to chemical properties and the influence on regulation of biosynthetic gene clusters in conjunction with the induction of transport systems by metal cations.

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