BioMetals (2006) 19:367–377
DOI 10.1007/s10534-005-3496-1

Interactions between iron availability, aluminium toxicity and fungal siderophores

Paul Illmer* & Rudolf Buttinger
*Institute of Microbiology, University of Innsbruck, Technikerstr. 25, A-6020, Innsbruck, Austria; *Author for correspondence (E-mail: Paul.Illmer@uibk.ac.at)*

Received 3 June 2005; accepted 24 September 2005

Key words: aluminium toxicity, siderophore, iron availability, Neurospora, Aspergillus, Hymenoscyphus

Abstract

The influence of iron, aluminium and of the combined application of both metals on microbial biomass and production of siderophores by three fungi (*Aspergillus nidulans*, *Neurospora crassa* and *Hymenoscyphus ericae*) were investigated. All three species showed a strong iron regulation and Al-sensitivity of siderophore biosynthesis although several differences remained species dependent. Inhibitory effects of Fe and Al on siderophore-production were additive and the higher binding capacity of siderophores towards iron could be compensated by a higher Al-availability. Although pH itself is also important for regulation of siderophore biosynthesis, an indirect effect of Al on siderophore production via an Al-induced pH decrease could be outlined. The toxic effects of Al resulting in a reduced biomass production were compensated by high Fe-availability, whereas the addition of DFAM, a bacterial siderophore, enhanced Al-toxicity.

Introduction

Al is the third most abundant element and the most abundant metal in the earth crust. Nevertheless, it is one of the very few macro-elements without biological function, which is probably due to the very strong toxicity towards all forms of life (Pina & Cervantes 1996). Al-toxicity is usually restricted to acidic conditions, which in turn complicates the distinction between the toxic effects of Al^{3+} and of H^{+} (Illmer & Erlebach 2003). Al-availability in soil and surface waters is limited due to its adsorption to mineral surfaces, and due to the formation of complexes with organic ligands such as humic substances and organic acids (van Hees *et al.* 2001). Widespread research concerning Al-toxicity began after the discovery that acid deposition leads to a reduction of soil pH and therefore to a mobilization of toxic Al. This is of great importance for the acidification of surface waters and soil (Illmer *et al.* 2003) and for the symptoms classed under the general term 'forest decline' (Johnson *et al.* 1981). Another interesting aspect of Al-toxicity is that several neurotoxicological and neurodegenerative symptoms like Alzheimer's disease have been attributed to Al-toxicity (Golub & Domingo 1996) and a distinct accumulation of aluminium in the nervous systems of patients suffering from these diseases have been determined (Savory *et al.* 1996). Although several toxic effects of Al are well established at a cellular level, the interactions between these mechanisms are still not understood (Pina & Cervantes 1996). Investigating the possible interactions between Al-toxicity and Fe-metabolism might throw some light on some unanswered questions (Yokel 2002).

Iron is an essential element for all forms of life except for members of the genus *Lactobacillus*. Although it is the fourth most abundant element in the earth crust (after oxygen, silicon and aluminium), the availability of iron to organisms is limited in O_2-rich environments. The solubility of Fe^{III} in aqueous solution at pH = 7 is about 10^{-17} M, which is far beyond the requirements of bacterial life (0.05–0.5 μM) (Martinez *et al.* 1990). Thus, very soon in the evolutionary development

of life, parallel to the increasing concentration of oxygen in the environment and the subsequent oxidation of the soluble ferrous to the insoluble ferric iron, micro-organisms were forced to respond to the decreasing iron availability by the production of specific microbial Fe^{III}-chelating agents, known as siderophores (Neilands 1993). About 500 different siderophores that are produced by fungi and aerobic and facultative anaerobic bacteria are currently known (Boukhalfa & Crumbliss 2002). Plants form analogous chelating agents (the so called phyto-siderophores), whereas no such structures are found in animal tissue (Neilands 1993). Chemically, most siderophores whose molecular weights do not exceed 1500 Dalton can be classed in two major groups: the catechols and the hydroxamates, with stability constants for complexation of $Fe(H_2O)_6^{3+}$ by the fully deprotonated ligand ranging from about 10^{23} to 10^{65} (Boukhalfa & Crumbliss 2002). The synthesis of siderophores is repressed by available iron (Eisendle *et al.* 2004), but the concentration of Fe necessary for inhibition depends on the type of the siderophore produced and on the respective organism (Haas 2003). A lot of work has been performed investigating the structure (Winkelmann & Drechsel 1997), and regulation of biosynthesis of siderophores (Haas 2003), and both siderophores and iron supply have been shown to be important factors for the virulence of several micro-organisms (Martinez *et al.* 1990; Sritharan 2000).

Despite the great number of investigations dealing with siderophores, the role of metals other than iron is not clear. How, if at all, these metals influence siderophore production and how the sensitivity towards these metals is influenced by siderophores is not known. Available studies have not come to a concurrent conclusion about these questions (Davis *et al.* 1971; Hu & Boyer 1996; Roy & Chakrabartty 2000; Rogers *et al.* 2001; Yokel 2002). In the few investigations dealing with this topic, fungi have been completely neglected although both free living and mycorrhizal fungi play a central role in all soil systems and although many of them are known to be potent siderophore producing organisms (Winkelmann & Drechsel 1997).

This current 'state of play' has led to two questions as a basis for the present investigation. Firstly, is quality and/or quantity of fungal siderophore production influenced by Al-availability and secondly, are the toxic effects of Al towards fungi influenced by the availability of iron and iron chelating substances (ICS)?

Material and methods

Media

All experiments were conducted in minimal medium (MM) at pH = 6.5 containing 1% glucose and 20 mM glutamine as C- and N-sources respectively (Oberegger *et al.* 2001). L-ornithine at a final concentration of 1 mM increased siderophore-production and was therefore added to the autoclaved medium in the form of a filter sterilized solution. The medium was not deferrated to enable microbial growth. However, the background concentration of Fe was low (0.05 μM) and the Fe was quickly spent (data not shown) so that siderophore-production was not hampered by this impurity. All experiments were conducted in Erlenmeyer-flasks made from polypropylene to avoid interactions of glass and metals. Solutions of $FeCl_3 \times 6H_2O$, $AlCl_3 \times 6\ H_2O$ and Desferal® (deferoxamine mesylate, DFAM) were applied in the form of filter sterilized solutions to reach the desired final concentrations in MM. Desferal® (Novartis Pharma AG, Basel, Switzerland) is produced by the fermentation of *Streptomyces pilosus* and is used in the therapy of patients with iron or aluminium overload. pH-variations were conducted using i) unbuffered MM where pH was initially adjusted with HCl and NaOH and ii) buffered MM using β-β-dimethylglutaric-NaOH-buffer for *Aspergillus nidulans* and *Hymenoscyphus ericae* and K-H-phtalate buffer and MES for *Neurospora crassa* (end-concentrations in MM 50 mM each).

Microorganisms and culture conditions

Three species – all of them belonging to the *Ascomycotes* were used within the present investigation: *Aspergillus nidulans*, which was described elsewhere in connection with siderophore production (Eisendle *et al.* 2004), *Neurospora crassa* (DSMZ No. 894) and *Hymenoscyphus ericae*, known to form ericoid mycorrhiza (Federspiel *et al.* 1991). Liquid MM was inoculated using

small cubes of fresh surface culture on MM-agar-plates and incubated on a horizontal shaker (200 rpm) at 30, 25 and 20 °C for *A. nidulans*, *N. crassa* and *H. ericae* respectively as these temperatures are generally recommended (e.g. by DSMZ) and led to high and reproducable biomass production. Depending on the actual experimental conditions it took the three micro-organisms about one (*A. nidulans*, *N. crassa*) and three (*H. ericae*) weeks respectively till stationary phase was reached and maximum biomass was produced.

Analysis

Microbial biomass was determined after filtration (Sartorius No. 11107, 0.45 µm) and drying at 80 °C. Supernant solutions were gathered and analyzed with respect to pH and redox potential (Metrohm 744 pH meter; equipped with Solitrode Pt 1000 and LL combined Pt-ring electrodes). Fe- and Al-content were determined using standard methods for GF-AAS (Hitachi Z-8200 Polarized Zeemann AAS). ICS were determined using the universal CAS test (Schwyn & Neilands 1987) and siderophores were analyzed with reversed phase HPLC (Haselwandter & Winkelmann 1998).

Results and discussion

In Figure 1 microbial growth, pH-change and production of ICS of the three micro-organisms are presented, indicating that *A. nidulans* and *N. crassa* reach maximum biomass within 1 week but *H. ericae* not before 25 days. The speed of growth was found to correspond with the incubation temperature used for the three micro-organisms studied. pH in solutions of all three micro-organisms decreased rapidly and showed a distinct secondary increase in the late phases of cultivation, a course which reflects the production of organic acids during growth phase and the beginning of autolytic processes at the end of incubation (Braga *et al.* 1999). Siderophore-production started very quickly in cultures of *A. nidulans* and *N. crassa* and reached a remarkable concentration after a few days – a time period in which *H. ericae* has not even started appreciable ICS-production at all. However, after 25 days the concentration of ICS in supernant solutions of *H. ericae* also reached a similar order of magnitude.

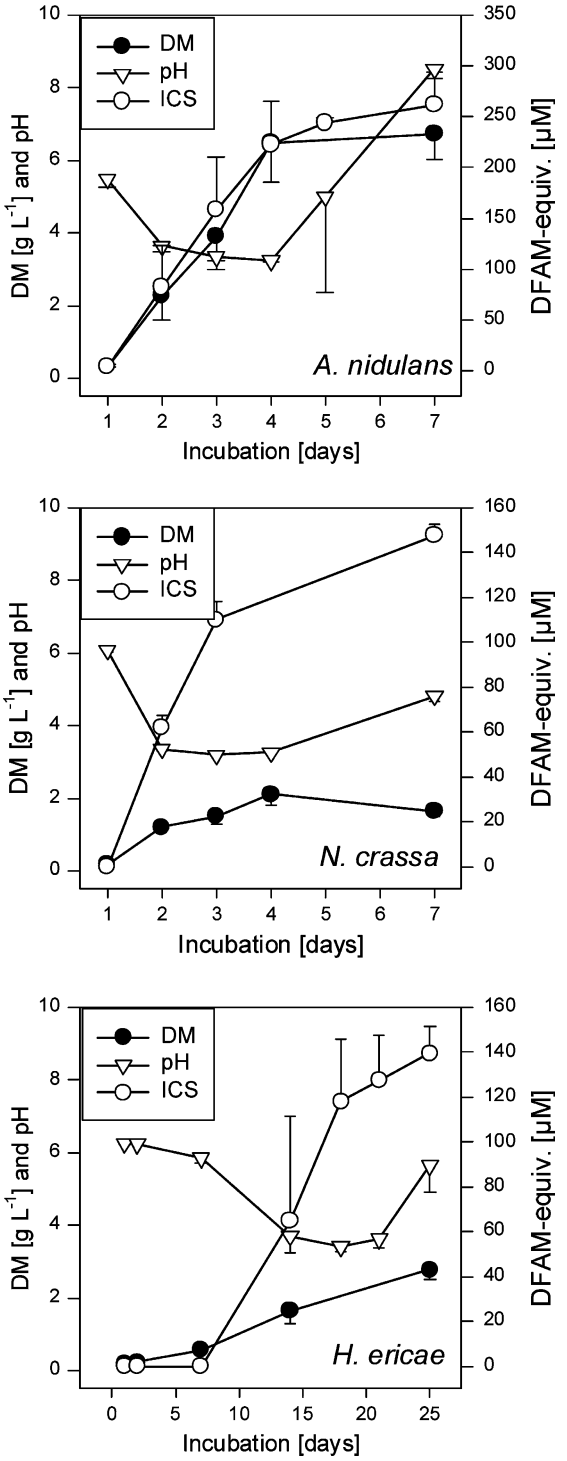

Figure 1. Changes in microbial biomass (DM, in g l^{-1}), pH and ICS (in µM DFAM-equivalent) during an incubation of *A. nidulans*, *N. crassa*, and *H. ericae* in MM.

Organic acids like oxalate and citrate (data not shown) and also fragments and decomposed parts of siderophores (Oberegger *et al*. 2001) produce positive signals in CAS-assay due to a decolourization of the blue complex. Thus ICS determined via the CAS-assay should not be called siderophores without further analysis. However, CAS-signal (expressed in DFAM-equivalents throughout the present study) is worth investigation as it reflects the biologically relevant sum of ICS. A precise analysis of supernant solutions using reversed phase HPLC clearly identified the main extra cellular siderophore substance of *A. nidulans*, *N. crassa* and *H. ericae* to be TAFC, coprogene and ferricrocin respectively, which corresponds with former studies (Federspiel *et al*. 1991; Haas 2003). So, although the quantity of siderophores was strongly influenced by the experimental conditions of the present investigation, the typical siderophore spectrum of the three micro-organisms did not change under all tested conditions.

Iron

Good growth in MM without additional Fe-application indicates that Fe was initially not a limiting factor for the growth of the three micro-organisms under investigation, but the background impurities of 0.05 μM must be kept in mind. Several authors give very different values regarding the minimum and optimum Fe-demand of micro-organisms. However, usually concentrations between 0.05 and 0.5 μM Fe which are frequently found as impurities in many media were found to be high enough to allow near-maximal growth (Martinez *et al*. 1990). While *A. nidulans* and *N. crassa* were not influenced in their maximum biomass production by different Fe-applications, *H. ericae* approximately doubled the produced dry matter at 1 μM Fe compared to the lowest level (Figure 2 insertion). Moreover, up to 1 μM Fe *H. ericae* also increased both the absolute and the relative (per biomass DM) ICS-production. Contrary, ICS-concentrations in supernant solutions and the amount of ICS produced per biomass decreased where the other two micro-organisms were concerned (Figure 2). The obviously higher Fe-demand of *H. ericae* connected with a lower sensitivity of ICS-biosynthesis against Fe (Figure 2) might be caused by an adaptation to acidic conditions, as *H. ericae* is often found in acidic soils with increased Fe-availability. *H. ericae* is also known to be the most important mycorrhizal endophyte of *Ericales* in these

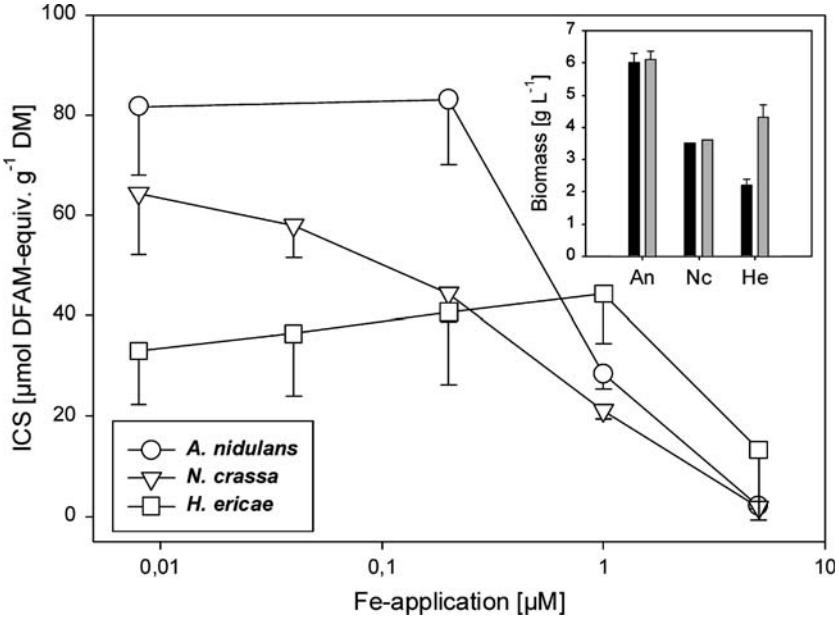

Figure 2. Effect of different Fe-concentrations on the production of ICS g^{-1} DM of *A. nidulans*, *N. crassa* and *H. ericae* at the end of the log phase. Insertion: Effect of the addition of 0 (black) and 1 (grey) μmol Fe l^{-1} MM on the biomass production of the three microorganisms at the end of the log phase.

habitats (Read 1996), thus it might be useful to maintain siderophore-production and Fe-acquisition at a high level.

To get at least an idea of the ratio of Fe^{3+}/Fe^{2+}, the redox potential in culture solutions was measured, because, like other metals, the availability of iron does not only depend on the availability of possible chelating and complex forming substances, but also on the pH and E_H (Babich & Stotzky 1980). E_H-values decreased from about 310 mV at $t=0$ down to about 95 and 105 mV in culture solutions of *A. nidulans* and *H. ericae*, whereas E_H remained unchanged in solutions of *N. crassa*. According to the connections $p\varepsilon = \log K + \log (Fe^{3+}/Fe^{2+})$ for $\log K = 13$ at 25 °C and $p\varepsilon = 1/0.059 \times E_H$, the ratio of Fe^{3+} to Fe^{2+} should sharply decrease in aqueous solutions from $\approx 10^{-8}$ to $\approx 10^{-12}$ when E_H decreases from 300 to 100 mV. Thus, redox potentials which are as low as those found in supernant solutions of *A. nidulans* and *H. ericae* and which in nature are usually connected to O_2-limited conditions should lead to a sharp increase in Fe^{2+}-availability (Ratering *et al.* 2000). Therefore, the necessity for high affinity uptake systems should decrease (Hantke 1987). Indeed, the concentration of ICS in supernatant solutions of *A. nidulans* and *H. ericae* at the end of the incubation period significantly correlated with E_H ($r=0.822$, $P<0.01$). The lack of an E_H-decrease in solution of *N. crassa* was surprising but might be connected with alternative (reductive) Fe-uptake systems known from this species (Winkelmann 1991). However, this aspect of Fe-availability needs more attention, both in explaining results already obtained and in further investigations.

Aluminium

Microbial growth was delayed when Al was added to MM, but where Al-concentration did not become too high (≤ 400 μM), the biomass production reached the level of the Al-free samples about 2 (*A. nidulans* and *N. crassa*) and 3 (*H. ericae*) days later, a phenomenon which is also known in connection with bacteria (Illmer & Erlebach 2003). Thus a couple of experiments were conducted in the low Al range allowing an equalization of microbial biomass, as we wanted to test a biomass independent influence of Al on siderophore-production. Despite the constant biomass, the concentrations of ICS in supernatant solutions changed significantly and this effect was species dependent (Figure 3): *N. crassa* and *H. ericae* were sensitive to Al, showing a complete inhibition of ICS-production at about 400 and 100 μM Al respectively, whereas ICS-production was found to increase in Al-applied culture solutions of *A.*

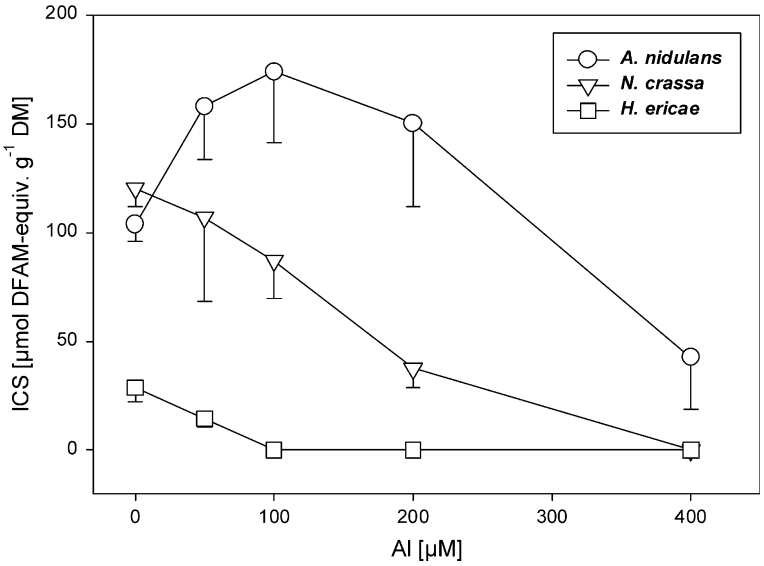

Figure 3. Effect of different Al-concentrations on the production of ICS g^{-1} DM of *A. nidulans*, *N. crassa* and *H. ericae* at the end of the log phase

nidulans up to a concentration of 100 μM Al. Hu & Boyer (1996) were also able to show that siderophore production increased in the presence of Al, when iron was a limiting factor. However, they could not find an Al-mediated induction of siderophore biosynthesis in cultures with sufficient iron which corresponds with our data. Roy & Chakrabartty (2000) were able to show that siderophore synthesis of *Rhizobium* sp. was increased by the addition of 100 μM Al^{3+}. However, it is not clear if the increase in ICS-production of *A. nidulans* should be interpreted as a positive response. It seems even more probable that *A. nidulans* increases ICS-production (i) to chelate and detoxify Al (Rogers *et al.* 2001; Yokel 2002), (ii) to improve Fe-nutrition which might be hampered through Al–Fe-competion (Zatta *et al.* 2002) or (iii) to respond to an Al-induced oxidative stress (Exley 2003). Remarkable analogous reactions towards Al which distinctly differ from a 'normal' sigmoid like reaction towards a stress factor are discussed in general and in connection with plant roots by (Barceló & Poschenrieder 2002). They assumed several possible explanations including the stimulation of various defense reactions and an improved Fe- and P-uptake.

pH

One difficulty in all investigations dealing with Al-toxicity is the discrimination between the effects of Al *per se*, and the effects of Al-induced pH-reductions. Using unbuffered nutrient solutions pH in culture solution changed dramatically but hardly predictably. Generally a higher starting pH led to a faster secondary increase but a proper connection between starting pH on the one hand and biomass- and ICS-production on the other was not detectable. To avoid these problems further experiments were conducted in buffered MM and led to more conclusive results. In buffered nutrient solutions pH-values remained remarkably constant over the whole period of investigation, indicating that neither the buffer capacity was exhausted nor the buffer itself metabolized. In these solutions a distinct pH-dependence of ICS-production was established. While *A. nidulans* and *N. crassa* had an optimal ICS-production at pH = 6.0 and did not produce any ICS at or below 4.5, the pH-optimum of *H. ericae* was at 5.5. At pH = 4.5, the ICS production of *H. ericae* was still about 80% of the maximum which again indicates a possible adaptation of *H. ericae* to acidic conditions. Recently, it was shown that pH plays a central role in regulating ICS-biosynthesis (Eisendle *et al.* 2004). The reason for this regulation is not yet clear, as it works parallel to the well established Fe-dependent regulation system. One possible explanation is a protective role of siderophore-downregulation at acidic conditions as with decreasing pH, many potential toxic metals become available and might easily be complexed and even taken up with siderophores (Hu & Boyer 1996). Thus, irrespective of the presence or lack of iron, it might be useful to keep the concentration of siderophores low to match high availability of toxic metals. Another interesting possibility is the assumed ecological role of siderophores connected with their antibiotic force. Because of the higher average pH-optima of bacteria they should play a less important role at low pH compared with at neutral pH-conditions. However, this explanation is inconsistent with the previously discussed adaptation of *H. ericae* to acidic conditions. But, as the pH-regulation was established with *A. nidulans*, it is still not known, if *H. ericae* reacts in a similar way. Indeed species-dependent differences regarding the influence of pH on siderophore biosynthesis exist (Eisendle *et al.* 2004).

A direct comparison between Al- and pH-effects on siderophore-production led to the results presented in Figure 4. Two levels of Al (200 and 400 μM) were used for these experiments as at least 400 μM was high enough to affect siderophore-production (but not the biomass) of *A. nidulans*, *N. crassa* and *H. ericae*. Once again the sensitivity of *A. nidulans* towards Al was low, that of *N. crassa* intermediate (so that we present these data in Figure 4) and that of *H. ericae* high. Al-concentrations of 200 and 400 μM led to pH-shifts in MM down to 6.18 and 6.10 respectively. These very same pH values were achieved by application of HCl, but had no effects on ICS-production, both on absolute concentrations and on amounts per biomass dry matter (Figure 4). Thus it seems that Al-effects on siderophore-production are caused by Al itself and not by the accompanying pH decrease. The distinction between Al- and pH-effects is for methodological reasons at least difficult, as Al^{3+} acts as a cationic acid with a pK_S for the first deprotonation of about 4.9 (Babich & Stotzky 1980). In a former

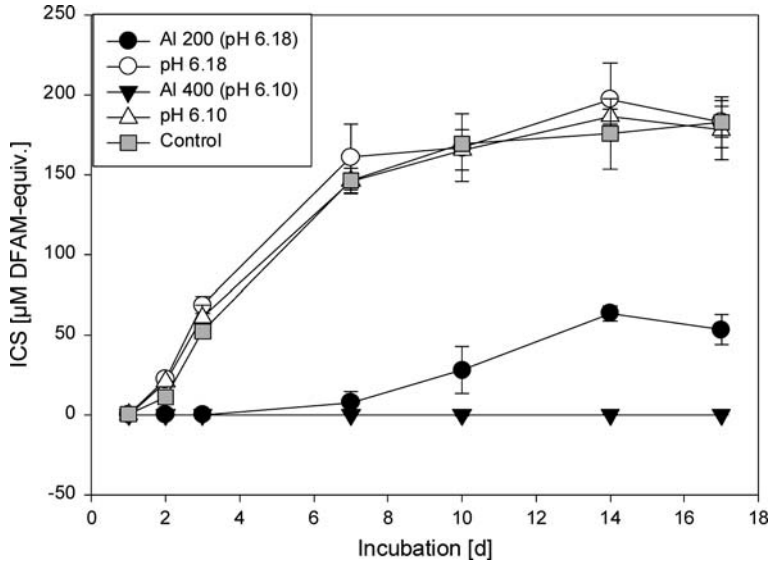

Figure 4. Effect of different Al-concentrations (200 and 400 µM) and the respective pH-levels (6.18 and 6.10) without Al on the production of ICS during an incubation of *N. crassa*. The control stands for ICS-production in MM without Al-addition at pH = 6.5

investigation (Illmer & Erlebach 2003), we were able to show, that acidity alone is only responsible for a small portion of the Al-effects – a result which was confirmed by the present data.

Iron and aluminium

The next logical step was to study the combined, biomass independent effects of Fe (1 µM for *A. nidulans* and *N. crassa* and 5 µM for *H. ericae*) and Al (300 µM for *A. nidulans*, 150 µM for *N. crassa* and 50 for *H. ericae*). These specific metal concentrations were used as they caused significant effects on ICS-production in preliminary experiments (see above and Figures 2 and 3). In Figure 5, *N. crassa* again stands in for the other two micro-organisms and clearly shows that the effects towards ICS-production were additive for the two metals under investigation (Figure 5a and b) whereas biomass was once again not affected at all (Figure 5b). Although Al caused a stronger inhibition of ICS-production than Fe did, the final extent was comparable and the differences in concentrations have to be considered (150 µM Al *versus* 1 µM Fe). Both metals together allowed only a very weak and very late ICS-production (Figure 5a). Results obtained with the other two organisms were very similar and confirmed our choice of a species dependent (and not a constant) metal concentrations for physiological investigations taking the different metal sensitivities into account.

Although siderophores are Fe-chelating agents, they are not really Fe-specific but they form the most stable complexes with Fe due to the ability of Fe to fill its 3d orbitals during hexadentate complex formation (Yokel 2002). However, several investigations, mainly dealing with structural aspects, found stable complexes of siderophores with metals including Al, Ga, Cu and Mo (Hu & Boyer 1996; Gaspar *et al*. 1999; Roy & Chakrabartty 2000, Rogers *et al*. 2001). As ionic radii of the trivalent cations Al^{III} (67.5 pm) and Fe^{III} (78.5 pm) are quite close these two metals should be able to substitute for one another (Albrecht-Gary & Crumbliss 1998). In fact, Al was found to bind similar tightly as Fe^{III} does which offers several medical application in treating metal-overload (Yokel 2002). Tam & McColl (1990) were able to show that Al distinctly interferes with various Fe and Ca binding organic ligands including catecholic and carboxylic ligands. Other studies established that Al-siderophore-complexes are very weak compared with those formed with Fe (Shenker *et al*. 1996). However, the ratios of stability constants usually found in the literature are in the range of about 150:1 (Gaspar *et al*. 1999) the same ratio of Al/Fe used in the experiments presented in Figure 4. Thus the higher binding stability of the Fe-siderophore-complex may be

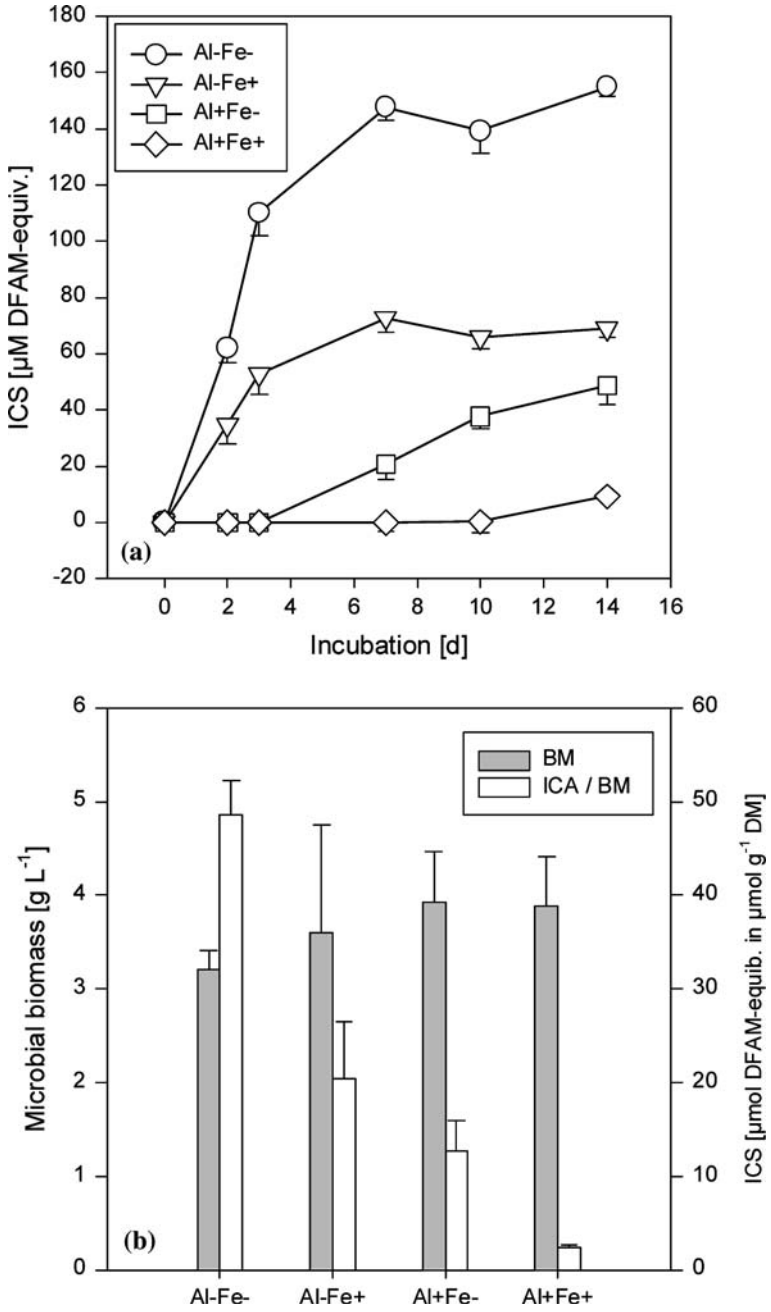

Figure 5. Effect of combined applications of Fe (0 and 1 µM) and Al (0 and 150 µM) on the production of ICS during an incubation of *N. crassa* (a) and on the production of biomass and ICS g^{-1} DM at the end of the log phase (b)

compensated by a higher availability of Al. As Al is ubiquitous and under acidic condition often present in quite large amounts (van Hees *et al.* 2001), this ratio of the metals might occur in natural environments as well so that a successful competition of Al with Fe for siderophores should be possible.

Deferoxamine mesylate, DFAM

In another set of experiments, we did not wait until the biomass production leveled in Al-applied and Al-free samples. Instead, we used a wider range of Al-levels and measured microbial biomass at $t=4$ for *A. nidulans* and *N. crassa*, and at $t=25$ for

H. ericae. At those particular times the respective organisms reached the end of the the exponential phase in Al-free MM. These experiments are summarized in Figure 6 where an interaction-diagram of a multi-factor ANOVA analysis of mean standardized biomass data of all three micro-organisms under investigation is presented. Without biomass equalization a strong inhibitory Al-effect became obvious even at Al-levels below 400 μM (Figure 6a). When the Fe concentration was increased to 10 μM (when no DFAM added), thus completely inhibiting siderophore biosynthesis, a striking ameliorative effect on biomass-production and a nearly complete compensation of the Al-effect became obvious (Figure 6b), findings which correspond with the conclusions of Middaugh *et al*. (2005). However, this ameliorative effect might be caused by an iron-induced avoidance of ICS-production leading to a hampered Al-uptake or alternatively by direct Fe–Al-interactions and competition for several binding sites (Zatta *et al*. 2002).

To clarify this, we included the application of DFAM (35 μmol DFAM l^{-1} MM) to enable a distinction between effects caused by the addition of Fe *per se* and effects caused by the Fe-induced inhibition of siderophore-biosynthesis. About 35 μM DFAM is a moderate concentration when compared to the amount of ICS actually produced by the three micro-organisms under investigation. Without Fe-application, DFAM resulted in a significantly reduced microbial biomass, which was probably caused by an increased, DFAM-mediated bioavailability of Al. Once again this points to a close connection between microbial siderophores and Al-toxicity. In contrast Rogers *et al*. (2001) found that vicibactin reduced the toxicity of Al by complexing it outside the cell. Davis *et al*. (1971) also found that a Sid^{-}-mutant of *Bacillus megaterium*, unable to produce schizokinen, was significantly more sensitive towards Al than the wild type was, again pointing to a protective role of siderophores. However, with the same bacterium Arceneaux *et al*. (1984) found a siderophore

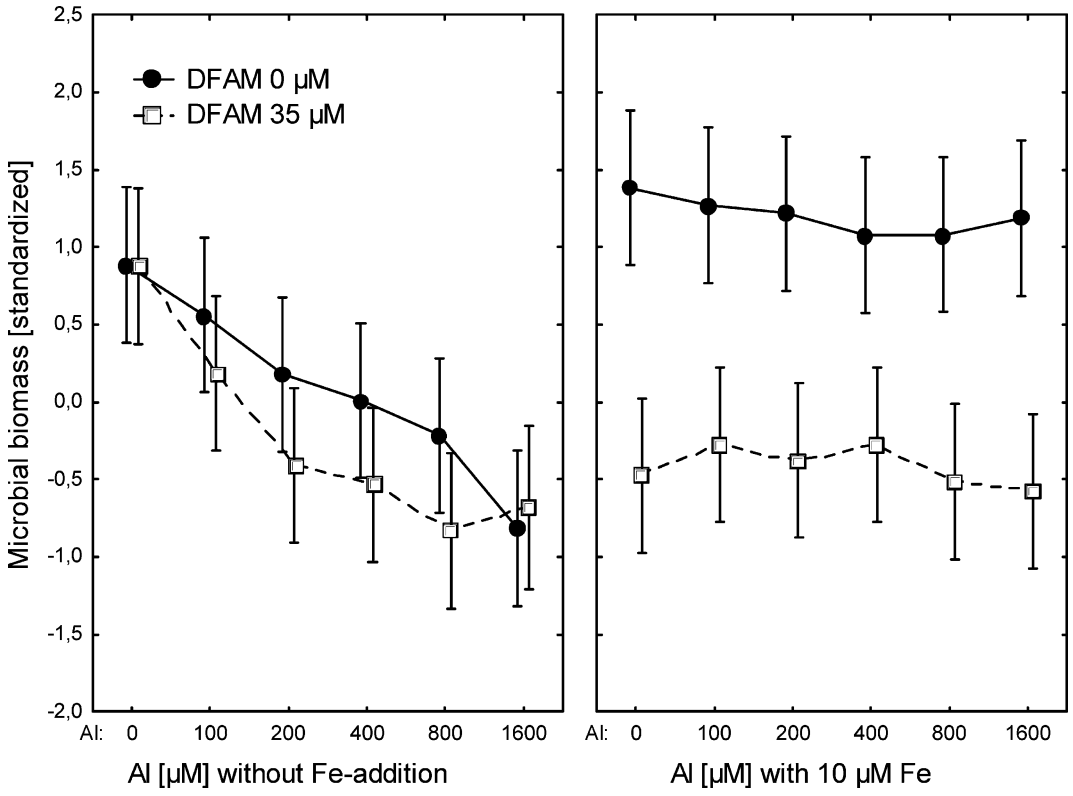

Figure 6. Interaction plot of a multi-factor ANOVA. Effect of Fe (0 and 10 μM) and DFAM (0 and 35 μM) together with different Al-concentrations (0–1600 μM) on the production of microbial biomass of *A. nidulans*, *N. crassa* and *H. ericae*. Biomass data of the respective organisms were standardized prior to calculation. Vertical bars give 95%-confidence intervals

mediated increase in Cu-toxicity. As stated above, comparable investigations with fungi are lacking and so the effects of Al on several siderophore deficient mutants (e.g. of *A. nidulans*) will be investigated soon in order to throw some light on this matter. Together with Fe (10 μM), the DFAM-application resulted in a very strong and Al-independent inhibition of biomass production (Figure 6b). This points to a toxicity of 10 μM Fe when applied together with siderophores, a combination which usually does not occur due to the strict regulation of siderophore-biosynthesis through Fe-availability. In the higher Al-range it may also be a combined effect of both Al- and Fe-toxicity comparable with those presented in Figure 4. Toxicity of iron is usually attributed to oxidative stress (Chamnongpol *et al*. 2002) and so a further similarity becomes obvious as oxidative stress is well established in connection with Al (Exley 2003).

To reconsider the questions mentioned at the beginning we conclude that (i) quantity but not quality of fungal siderophores are distinctly influenced by Al-availability and (ii) that there are strong interactions between Al-toxicity and concentrations of Fe and ICS. Whereas both Fe and Al inhibit ICS-synthesis, the toxic effects of Al on microbial biomass are reduced by Fe and enhanced by ICS.

Acknowledgements

This work was supported by the Austrian Science Foundation (FWF P 15018). We are grateful to H. Haas and K. Haselwandter for kindly providing us with *A. nidulans* and *H. ericae* respectively.

References

Albrecht-Gary AM, Crumbliss AL. 1998 Coordination chemistry of siderophores: thermodynamics and kinetics of iron chelation and release. In: Sigel A, Sigel H, eds. *Iron Transport and Storage in Microorganisms, Plants, and Animals*. New York: Marcel Dekker; pp. 239–327.

Arceneaux JEL, Boutwell ME, Byers BR. 1984 Enhancement of copper toxicity by siderophores in *Bacillus megaterium*. *Antimicrob Agents Ch* **25**, 650–652.

Babich H, Stotzky G. 1980 Environmental factors that influence the toxicity of heavy metal and gaseous pollutants to microorganisms. *Crit Rev Microbiol* **8**, 99–145.

Barceló J, Poschenrieder C. 2002 Fast root growth response, root exudates, and internal detoxification as clues to the mechanisms of aluminium toxicity and resistance: a review. *Environm Experim Bot* **48**, 75–92.

Boukhalfa H, Crumbliss AL. 2002 Chemical aspects of siderophore mediated iron transport. *BioMetals* **15**, 325–339.

Braga GUL, Destéfano RHR, Messias CL. 1999 Protease production during growth and autolysis of submerged *Metarhizium anisopliae* cultures. *Revista de Microbiologia* **30**, 107–113.

Chamnongpol S, Dodson W, Cromie MJ, Harris ZL, Groisman EA. 2002 Fe(III)-mediated cellular toxicity. *Mol Microbiol* **45**, 711–719.

Davis WB, McCauley MJ, Byers BR. 1971 Iron requirements and aluminium sensitivity of an hydroxamic acid-requiring strain of *Bacillus megaterium*. *J Bacteriol* **105**, 589–594.

Eisendle M, Oberegger H, Buttinger R, Illmer P, Haas H. 2004 Biosynthesis and uptake of siderophores is controlled by the PacC-mediated ambient-pH regulatory system in *Aspergillus nidulans*. *Eukaryotic Cell* **3**, 561–563.

Exley C. 2003 The pro-oxidant activity of aluminium. *Free Radical Bio Med* **36**, 380–387.

Federspiel A, Schuler R, Haselwandter K. 1991 Effect of pH, L-ornithine and L-proline on the hydroxamate siderophore production by *Hymenoscyphus ericae*, a typical ericoid mycorrhizal fungus. *Plant Soil* **130**, 259–261.

Gaspar M, Grazina R, Bodor A, Farkas E, Santos MA. 1999 Siderophore analogues: a new macrocyclic tetraamine tris(hydroxamate) ligand; synthesis and solution chemistry of the iron(III), aluminium(III) and copper(II) complexes. *J Chem Soc, Dalton Trans* **5**, 799–806.

Golub MS, Domingo JL. 1996 What we know and what we need to know about developmental aluminium toxicity. *J Toxicol Environm Health* **48**, 585–597.

Haas H. 2003 Molecular genetics of fungal siderophore biosynthesis and uptake: the role of siderophores in iron uptake and storage. *Appl Microbiol Biotechnol* **62**, 316–330.

Hantke K. 1987 Ferrous iron transport mutants in *Escherichia coli* K12. *FEMS Microbiol Lett* **44**, 53–57.

Haselwandter K, Winkelmann G. 1998 Identification and characterization of siderophores of mycorrhizal fungi. In: Varma A, eds. *Mycorrhiza Manual*. Springer:Heidelberg: pp. 243–254.

Hu X, Boyer GL. 1996 Siderophore-mediated aluminium uptake by *Bacillus megaterium* ATCC 19213. *Appl Environm Microbiol* **62**, 4044–4048.

Illmer P, Erlebach C. 2003 Influence of Al on growth, cell size and content of intracellular water of *Arthrobacter* sp. PI/1–95. *Anton Leeuw Int J G* **84**, 239–246.

Illmer P, Obertegger U, Schinner F. 2003 Microbiological properties in acidic forest soils with special consideration of KCl extractable Al. *Water Air Soil Poll* **148**, 3–14.

Johnson NM, Driscoll CT, Eaton JS, Likens GE, Mcdowell WH. 1981 'Acid rain', dissolved aluminium and chemical weathering at the Hubbard Brook Experimental Forest, New Hampshire. *Geochim Cosmochim Acta* **45**, 1421–1437.

Martinez JL, Delgado-Iribarren A, Baquero F. 1990 Mechanisms of iron acquisition and bacterial virulence. *FEMS Microb Rev* **75**, 45–56.

Middaugh J, Hamel R, Jean–Baptiste G, Beriault R, Chenier D, Appanna VD. 2005 Aluminium triggers decreased aconitase activity via Fe-S cluster disruption and the overexpression of isocitrate dehydrogenase and isocitrate lyase. *J Biol Chem* **280**, 3159–3165.

Neilands JB. 1993 Siderophores. *Arch Biochem Biophys* **302**, 1–3.

Oberegger H, Schoeser M, Zadra I, Abt B, Haas H. 2001 SREA is involved in regulation of siderophore biosynthesis, utilization and uptake in *Aspergillus nidulans*. *Mol Microbiol* **41**, 1077–1089.

Pina RG, Cervantes C. 1996 Microbial Interaction with aluminium. *BioMetals* **9**, 311–316.

Ratering S, Schnell S. 2000 Localization of iron-reducing activity in paddy soil by profile studies. *Biochem* **48**, 341–365.

Read DJ. 1996 The structure and function of the ericoid mycorrhizal root. *Annals Bot* **77**, 365–374.

Rogers NJ, Carson KC, Glenn AR, Dilworth MJ, Hughes MN, Poole RK. 2001 Alleviation of aluminium toxicity to *Rhizobium leguminosarum* bv. *viciae* by the hydroxamate siderophore vicibactin. *BioMetals* **14**, 59–66.

Roy N, Chakrabartty PK. 2000 Effect of aluminium on the production of siderophore by *Rhizobium* sp. (*Cicer arietinum*). *Curr Microbiol* **41**, 5–10.

Savory J, Exley C, Forbes WF, Huang Y, Joshi JG, Kruck T, McLachlan DRC, Wakayama I. 1996 Can the controversy of the role of aluminium in Alzheimer's disease be resolved? What are the suggested approaches to this controversy and methological issues to be considered? *J Toxicol Env Health* **48**, 615–635.

Schwyn B, Neilands JB. 1987 Universal chemical assay for the detection and determination of siderophores. *Anal Biochem* **160**, 47–56.

Shenker M, Hadar Y, Chen Y. 1996 Stability constants of the fungal siderophore rhizoferrin with various microelements and calcium. *Soil Sci Soc Am J* **60**, 1140–1144.

Sritharan M. 2000 Iron as a candidate in virulence and pathogenesis in mycobacteria and other microorganisms. *World J Microb Biot* **16**, 769–780.

Tam S.-C, McColl JG. 1990 Aluminium- and calcium-binding affinities of some organic ligands in acidic conditions. *J Environm Qual* **19**, 514–520.

vanHees PAW, Lundström US, Danielsson R, Nyberg L. 2001 Controlling mechanisms of aluminium in soil solution-an evaluation of 180 podzolic forest soils. *Chemosphere* **45**, 1091–1101.

Winkelmann G. 1991 Specifity of iron transport in bacteria and fungi. In: Winkelmann G, eds. *Handbook of Microbial Iron Chelates*. Boca Raton: CRC Press, pp. 65–105.

Winkelmann G, Drechsel H. 1997 Microbial siderophores. In: Kleinkauf H, Döhren Hvon, eds. *Products of Secondary Metabolism*. 7 Weinheim: VCH Verlagsgesellschaft, pp. 199–246.

Yokel RA. 2002 Aluminium chelation principles and recent advances. *Coord Chem Rev* **228**, 97–113.

Zatta P, Kiss T, Suwalsky M, Berthon G. 2002 Aluminium(III) as a promoter of cellular oxidation. *Coord Chem Rev* **228**, 227–284.