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Ecology of siderophores with special reference to the fungi

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Abstract Ecology of siderophores, as described in the present review, analyzes the factors that allow the production and function of siderophores under various environmental conditions. Microorganisms that excrete siderophores are able to grow in natural low-iron environments by extracting residual iron from insoluble iron hydroxides, protein-bound iron or from other iron chelates. Compared to the predominantly mobile bacteria, the fungi represent mostly immobile microorganisms that rely on local nutrient concentrations. Feeding the immobile is a general strategy of fungi and plants, which depend on the local nutrient resources. This also applies to iron nutrition, which can be improved by excretion of siderophores. Most fungi produce a variety of different siderophores, which cover a wide range of physico-chemical properties in order to overcome adverse local conditions of iron solubility. Resource zones will be temporally and spatially dynamic which eventually results in conidiospore production, transport to new places and outgrow of mycelia from conidiospores. Typically, extracellular and intracellular siderophores exist in fungi which function either in transport or storage of ferric iron. Consequently, extracelluar and intracellular reduction of siderophores may occur depending on the fungal strain, although in most fungi transport of the intact siderophore iron complex has been observed. Regulation of siderophore biosynthesis is essential in fungi and allows an economic use of siderophores and metabolic resources. Finally, the chemical stability of fungal siderophores is an important aspect of microbial life in soil and in the rhizosphere. Thus, insolubility of iron in the environment is counteracted by dissolution and chelation through organic acids and siderophores by various fungi.

Keywords Iron · Siderophores · Fungi · Yeast · Mycorrhiza · Ecology

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Introduction

Ecology deals with both, organisms and environment and can be regarded as the studies of factors that control the distribution and abundance of organisms (MacArthur 2006). It simply summarizes the properties that are required to survive in a natural environment. The topic ecology of siderophores is likewise an analysis of microorganisms in their environment under iron-limiting conditions and their siderophores produced. Since the first observation of ferrichrome (Neilands 1952) and coprogen (Hesseltine et al. 1952) we

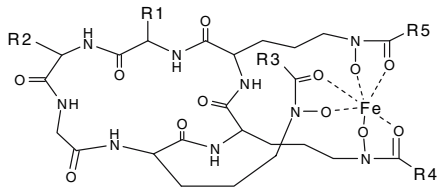
know that low-molecular weight (<1500 Da) ferric-specific chelates, collectively called siderophores, are produced under iron limitation which according to their iron-binding ligands are classified either as hydroxamates, catecholates, hydroxycarboxylates or mixed functional compounds (Winkelmann 1986; Drechsel and Winkelmann 1997). Contrary to most other essential metal ions entering microbial cells, iron(III) needs to be solubilized by binding to siderophores which then can enter microbial cells via specific siderophore transport systems. This is a general mechanism of aerobic microorganisms with only few exceptions where iron is substituted by other essential ions such as manganese in lactobacteria and some pathogens which can mobilize ferric iron directly from iron-containing eukaryotic host proteins, like transferrin, lactoferrin, ferritin or from heme using a heme oxigenase. Thus, although iron is abundant, it is not easily available and therefore ecology is a description of the various factors that influence iron mobilization in nature. This review will briefly discuss some important ecological aspects of siderophores and discuss their molecular design for the environment. While the general topic of metal ions in fungi has been published previously (Winkelmann and Winge 1994; Winkelmann 2001, 2002), the topic ecology of siderophores has only recently been introduced (Winkelmann 2004) with regard to various microbial communities in a chapter of a previous book on Iron Transport in Bacteria (Crosa et al. 2004). The present review will focus in more detail on the fungal siderophores which due to their wide-spread occurrence in natural environments deserve special attention.

Main fungal siderophores

The number of structurally different fungal siderophores has increased by now to about 100–150 with ferrichromes, coprogens, fusarinines and polycarboxylates as the major siderophore classes. The ferrichrome family (Fig. 1) consists of at least 20 structurally different hexapeptides including several asperchromes and other derivatives (Jalal and van der Helm 1981; Jalal et al. 1984) Besides ferrichromes, ester-containing coprogens and fusarinines are produced so that one fungal organisms may produce a set of siderophores covering a wide range of physico-chemical properties. Ferrichromes show either variation in the sequence of amino acids (glycine, serine, alanine) in the peptide ring or in the hydroxamic acid residues (acetic acid, cis- and *trans*-anhydromevalonic acid, methylglutaconic acid or malonic acid). The tripeptide sequence orn-orn-orn is always present. Most of these ferrichromes have been structurally elucidated during 1960–1970 by the swiss group of Keller-Schierlein and Zähner in Zürich and by Neilands and Emery in Berkeley and Logan. Also crystal structures of ferrichrome and ferrichrome A (van der Helm et al. 1980) have subsequently been solved so that the ferrichromes were among the best known natural compounds at that time.

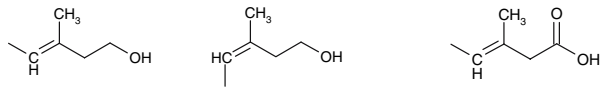
Fungi in the environment

The complexity of a fungal organism is ilustrated in Fig. 2, which represents an electron microscopic view (magnification 25 000) of a thin section of a hyphal tip of *Neurospora crassa*. This multiple-compartment system shows vacuoles, mitochondria, secretion vesicles, cell wall and cell membranes which all are involved in iron metabolism, siderophore excretion, storage and transport. Fungi are generally immobile organisms that need to exploit and explore their environment. Finding suitable resources for growth is the main goal of a mycelium that spreads out by developing a branching system. Foraging occures generally at the growing hyphal tips, which show the highest metabolic acitivity. This also applies to iron metabolism and transport of siderophores. We therefore always used freshly prepared germ tubes grown from conidiospores for our kinetic studies of iron transport. In fungi (Wiebe and Winkelmann 1975). Bacterial growth may be sometimes similar to fungal growth when colonies on surfaces or biofilms are produced. However, as soon as nutrient limitation proceeds in a natural environment bacterium can escape the immobile life of a colony and move as

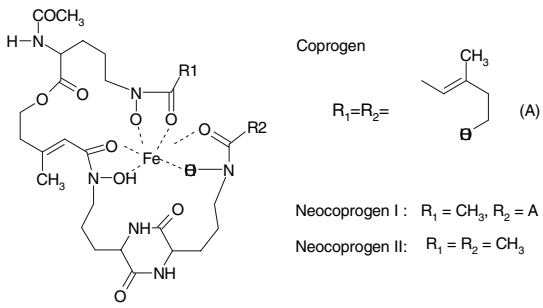


Ferrichrome	$R_1 = R_2 = H$	$R_3 = R_4 = R_5 = CH_3$
Ferricrocin	$R_1 =$ H, $R_2 = CH_2OH$	$R_3 = R_4 = R_5 = CH_3$
Ferrichrysin	$R_1 = R_2 = CH_2OH$	$R_3 = R_4 = R_5 = CH_3$
Asperchrome D1	$R_1 = R_2 = CH_2OH$	$R_3, R_4, R_5 =$ (2 CH_3, 1A)
Asperchrome B1	$R_1 = R_2 = CH_2OH$	$R_3, R_4, R_5 =$ (1 CH_3, 2A)
Ferrirubin	$R_1 = R_2 = CH_2OH$	$R_3 = R_4 = R_5 = A$
Ferrirhodin	$R_1 = R_2 = CH_2OH$	$R_3 = R_4 = R_5 = B$
Ferrichrome A	$R_1 = R_2 = CH_2OH$	$R_3 = R_4 = R_5 = C$

A **B** **C**



Ferrichromes



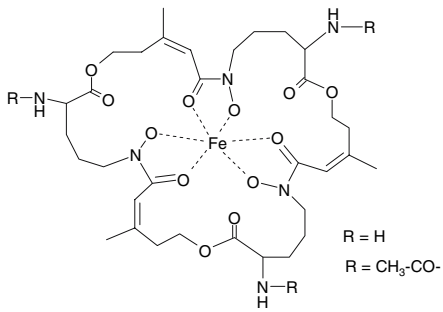
Coprogen

$R_1=R_2=$ (A)

Neocoprogen I : $R_1 = CH_3$, $R_2 = A$

Neocoprogen II: $R_1 = R_2 = CH_3$

Coprogens



R = H

$R = CH_3$-CO-

Fusigen (R = H) and Triacetylfusarinine C (=Triacetylfusigen) R = CH_3-CO-

Fig. 1 Structures of fungal siderophores

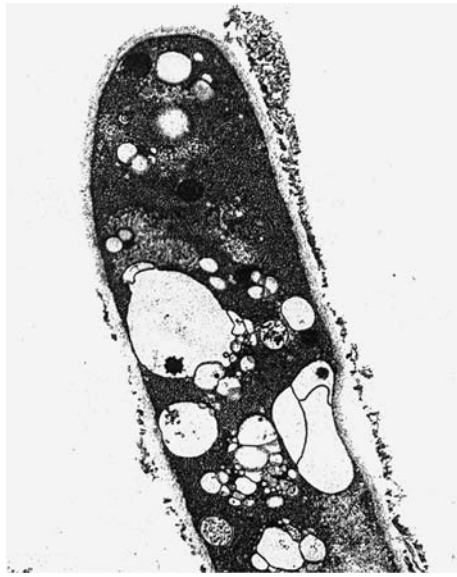


Fig. 2 Electron micrograph of a thin section of a hyphal tip of *Neurospora crassa* grown under iron-deficient conditions. In order to visualize the cell wall ferritin was added. The hypa contains large vacuoles, secretion vesicles, mitochondria and other compartments

free-living cells. Therefore, the feeding ecology of fungi greatly differ from that of bacteria.

Siderophores in soil

The chemical stability of siderophores is a precondition for their extracellular function. Among the tris-hydroxamate siderophores the ferrichromes have received the highest level of structural development as they consist of cyclic hexapeptides being largely resistant to environmental degradation. It is interesting to note that ferrichromes are the predominant siderophores of Aspergillus and Penicillium strains living in decaying organic materials. When discussing chemical stability of siderophores, we should be aware that decaying plant cells release a bunch of various hydrolases and proteases, which may affect the lifetime of certain siderophores. Also competing bacteria of the genus Bacillus and Streptomyces are well known for their exoenzymes, like esterases, lipases and proteases. Although no systematic studies exist on the enzymatic degradation of all kinds of siderophores, it can be assumed that the ferrichromes are generally resistant to rapid enzymatic degradation, especially when present as ferric complexes. Ferrichrome, ferricrocin, ferrichrysin and their N-acyl derivatives ferrirubin, ferrirhodin and ferrichrome A are relatively persistant in soil. Young fine roots or root hairs of plants are rapidly colonized by various bacteria in soil (Fig. 3), but preferentially by bacteria of the genus Pseudomonas, which utilize all kinds of low-molecular exudates from plant roots. As Psudomonads have their own efficient siderophore system, competition for iron is generally not a problem for both organisms, although certain strains of bacteria may cause iron-deficiency symptoms on plants growing under low-iron stress. When growth proceeds the root of most plants develop a mutualistic association with certain fungi which is called mycorrhiza (Haselwandter and Winkelmann 1998). For example, roots of nearly all trees in temperate climates contain an ectomycorrhizal symbiosis (Fig. 4). This tight assossiation between fungi and plants is an evolutionary old symbiosis, which may have already been established when plants started to colonize the land surface. The siderophore of the ectendo-mycorrhizal fungus *Wilcoxina rehmii* and the ectomycorrhizal fungus *Cenococcum geophilum* have been identified as ferricrocin (Prabhu et al. 1996; Haselwandter and Winkelmann 2002). *C. geophilum* belongs to the ascomycetes which may explain the biosynthesis of ferricrocin as a major siderophore. Most other ectomycorrhial fungi

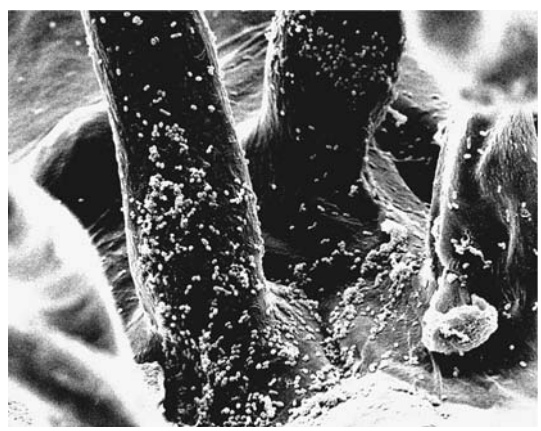


Fig. 3 Scanning electron micrograph of young fine roots from *Pinus sylvestris* containg attached bacteria

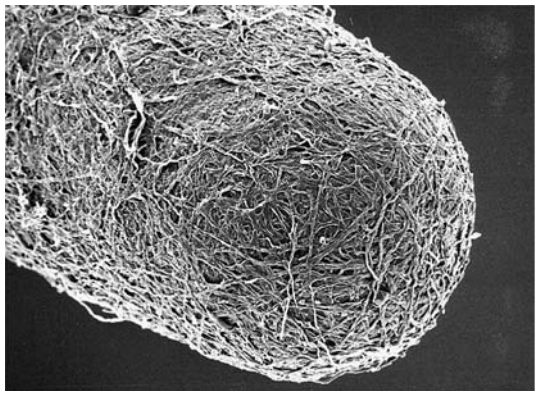


Fig. 4 Scanning electron micrograph of an ectomycorrhizal mantel of a rootlet. The root is completely covered with hyphae, which may further extend beyond the roots into the bulk soil

belong to the basidiomycetous fungi for which siderophores have not been described so far. However, a variety of ericoid mycorrhizal fungi have also been shown to produce ferricrocin in addition to fusigen (Haselwandter et al. 1992) which underlines the wide spread occurrence of ferricrocin in the rhizosphere of plants and in soil. It confirms the ecological fitness of ferrichrome compounds in a habitat where a variety of bacterial decomposeres are present. A novel siderophore, basidiochrome, (Fig. 5) has recently been isolated from mycorrhizal fungi of orchid roots, which belong to the genera *Ceratobasidium* and *Rhizoctonia* (Haselwandter et al. 2006).

Observations in swedisch podzolic forrest soils have revealed a siderophore concentration of

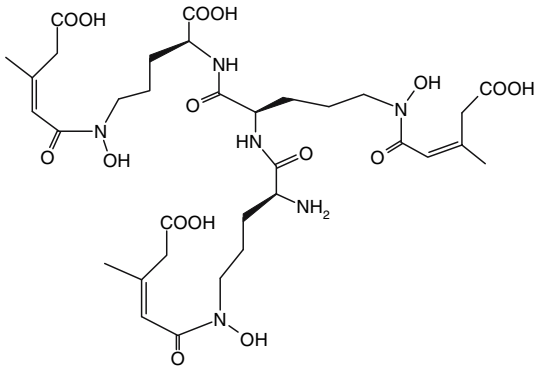


Fig. 5 Basidiochrome, a novel trishydroxamate siderophore from orchidaceous mycorrhizal fungi of *Ceratobasidium* and *Rhizoctonia* spp

2–12 nM, identified as ferrichrome and ferricrocin (Essén et al. 2006), although in earlier measurements using a microbial assay (*M. flavescens* JG9) somewhat higher amounts (28–279 nM) were detected probably due to the presence of additional amounts of ferrioxamines produced by streptomycetes (Powell et al. 1980, 1982; Szaniszlo et al. 1981). Soil ferrichrome and ferricrocin may originate from mycorrhizal fungi at the root surface of forrest trees which remain bound to humic matrix and soil particles. We recently showed that an ectomycorrhizal fungus *Cenococcum geophilum*, having a global distribution and a broad host range, produced ferricrocin as a major siderophore (Haselwandter and Winkelmann 2002). Thus, ferricrocin is certainly a wide-spread siderophore in forrest solis. On the other hand, Aspergillus strains of the fumigatus group (Diekmann and Krezdorn 1975) and a variety of other soil ascomycetes may occur in the upper layer of soils when plant material is present as a substrate. Therefore it seems questionable whether or not the mycorrhiza fungi are the only hydroxamate producers in the upper soil layer.

An important question is whether or not bacterial and fungal siderophores can be utilized by plants. Early reports suggested that iron from hydroxamates (ferrioxamine B) is transferred to the plant via solubilization and/or subsequent reduction at the rhizosphere (Cline et al. 1984). A similar conclusion was drawn from experiments with ^{55}Fe-siderophores from Pseudomonas (Bar-ness et al. 1991) showing that dicots acquire iron from pseudobactin by a reduction mechanism. Later studies using rhizospere microorganisms revealed that the bacterial siderophores like pseudobactins and ferrioxamines were inefficient as Fe sources. It was concluded that microorganisms are involved in degradation processes of microbial siderophores, as well as in competition for Fe with higher plants (Bar-Ness et al. 1992). Although rapid degradation of pseudobactin (now collectively called pyoverdins) seem to be unlikely, since alternate D- and L-configuration of amino acids in the peptide chain make them very resistant to proteases, ligand exchange mechanisms between pyoverdins and phytosiderophores seem to be a more attractive explanation for iron nutrition in gramineous plants (Yehuda et al. 1996). We

have shown for example that coprogen, produced by *Penicillium chrysogenum*, is easily split at the ester bond under alkaline conditions or by esterases to give dimerum acid and fusarinine which both in their ferric forms supported growth of dicots and monocots (Hördt et al. 2000). This suggested that due to a lower reduction potential mono- and dihydroxamates are better iron sources for plants than trishydroxamates. This seemed to be also true when ferric mono- and dihydroxamtes were applied as an foliar iron supply to plants (Fernandéz et al. 2004, 2005). The better iron donation properties of mono- and dihydroxamates compared to trishydroxamates may correlate well with the role of plant (leaf)plasma membrane-bound ferric-chelate reductase (Brüggemann et al. 1993) which reduces ferric citrate and Fe-EDTA in a NADH-dependent mode.

Although in general siderophores seem to be very resistant in soil, bacteria of the genus *Azospirillum* are able to degrade ferrioxamines when present as iron-free compounds (desferrioxmines) in pure culture (Winkelmann et al. 1996, 1999). This is surprising as accumulation of desferrioxamines in soil is unlikely. However, the rhizosphere is different and may contain various producers of desferrioxamines, as for example *Erwinia herbicola* species, so that local degradation of desferrioxamines by plant-associated *Azospirillum irakense* may occur directly in the neighbourhood of the roots where iron may be largely absent. These few examples show that siderophores of the ferrichrome family are chemically relatively stable in soil and are with few exceptions (Warren and Neilands 1965; Villavicencio and Neilands 1965) not easily degraded by microorganisms living in the same habitat. The persistance of ferrichromes in soil has the advantage that other microorganisms may profit from their presence. This opens the possibility of a succession of different bacteria and fungi to rapidly overgrow the preceeding culture. Soil-borne fungi like *Botrytis cinerea* (Konetschny-Rapp et al. 1988), *Gliocladium virens* (Jalal et al. 1986), *Trichoderma* (Anke et al. 1992) and soil yeasts of the Lipomycetaceae (van der Walt et al. 1990) have been shown to produce and utilize ferrichromes. Also *Escherichia coli*, which is unable to synthesize ferrichrome, possesses a FhuA receptor for uptake of ferrichromes. The same applies for the related enterobacterial genera *Enterobacter*, *Erwinia, Pantoea* and Yersinia. Obviously, evolution has equiped many bacteria to internalize the most common fungal siderophores. The reverse is true for the uptake of bacterial siderophores by fungi. Thus ferrioxamines and enterobactin can be utilized by certain fungi, like *Saccharomyces* and *Aspergillus* (Lesuisse et al. 1998; Heymann et al. 2000a; Haas 2003), The evolution of siderophores is still unknown but competition for iron is so essential that the development of siderophores and their cognate transport systems has influenced each other in a sense that when structural novel siderophores appeared, a subsequent utilization by other competing microorganisms followed.

Properties of siderophores

Everybody working in the laboratory with agar plates seeded with bacteria knows that fungal contaminants like Aspergillus and Penicillium strains may finally overgrow the bacterial colonies. This is in part due to the rapid growth of the mycelia especially at low water activity but also due to the excretion of various siderophores with all kinds of solubility properties. For example the lipophilic ferrirubin and ferrirhodin are often found together with ferrichrome, ferricrocin and ferrichrysin. as shown by a thin layer chromatogram of different siderophores from *Aspergillus* and *Penicillium* strains (Fig. 6). The thin layer plate consisted of silica gel that was run with a mixture of dichloromethane-methanol-water (70:30:4) as an eluent. The three upper spots on the right side show the lipophilic triacetylfusarinine C, followed by a line of ferrichromes (mainly ferrichrome or ferricrocin), two big spots of coprogen and ferrirubin in the middle and a variety of charged siderophores, like fusarinines, ferrichrome A, near the bottom at the start line. Modern HPLC separation (Fig. 7) allows a more detailed analysis of the various fungal siderophores. Thus the whole spectrum of lipophilicity is covered by the produced fungal siderophores. Also in the *Aspergillus fumigatus* group ferricrocin is often

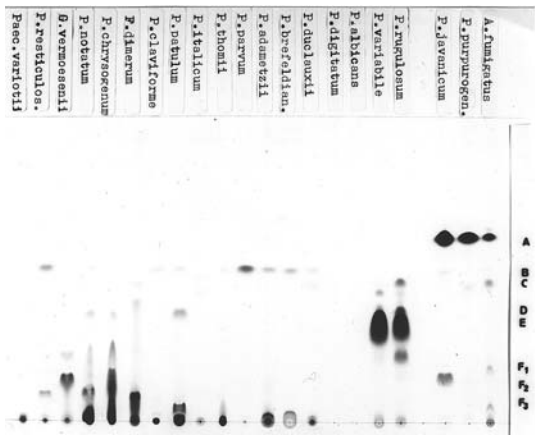


Fig. 6 Thin layer chromatogram of fungal siderophores isolated from low-iron cultures of *Aspergillus*, *Penicillium*, *Fusarium* and *Paecilomyces* strains using a silicagel plate and dichloromethane-methanol-water (70:30:4) as an eluent

accompanied by the more lipophilic triacetylfusarinine C. Thus, producing siderophores with a wider range of physico-chemical properties seems to by adventageous. While most ferrichromes are uncharged molecules, some of them like ferrichrome A have three negative charges under neutral conditions, which might influence the adsorption to positive charges at the cell wall. Ferrichrome and ferrichrome A are both produced by fungi of the Ustilaginales. *Ustilago*

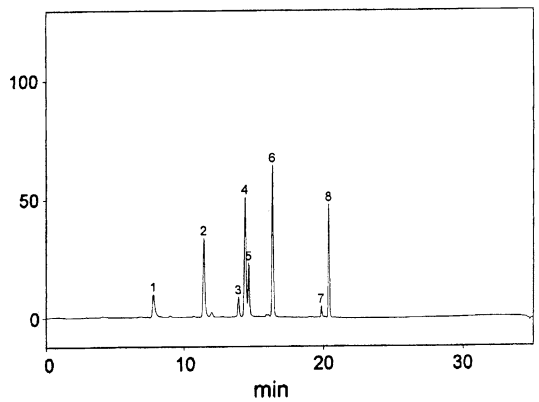


Fig. 7 HPLC chromatogram of selected fungal siderophores separated on a reversed phase (C18) column using an acetonitrile-water (0.1% TFA) gradient and a detector wavelength of 435 nm. Peak numbers are as follows: 1 Fe-dimerum acid, 2 neocoprogen II, 3 fusigen (linear), 4 fusigen (cyclic), 5 neocoprogen I, 6 coprogen, 7 triacetylfusarinine C (linear), 8 triacetylfusarinine C (cyclic)

maydis is a plant pathogen, which may cause serious losses in maize production. Emery and coworkers studied uptake of ferrichrome and ferrichrome A in *U.spaerogena* by using spectroscopic (EPR) techniques and concluded that uptake of ferrichrome A is dependent on a reduction process. Thus besides uptake of ferrichrome as an intact molecule, there was uptake of iron from ferrichrome A via reduction at the cell membrane (Ecker et al. 1982). This shows that besides solubility the mode of action for iron delivery to the cells may be affected by structural changes of siderophores. Emery also found uptake of the non-reducible gallium-desferrichrome A complex, suggesting that a certain but smaller amount of the intact ferrichrome A is transported into the cells of *U. sphaerogena*. He concluded that the slow uptake of ferrichrome A was superimposed by a massive reduction at the membrane surface. Although these results were confusing and were not fully understood, Emery (1987) suggested that a reductive iron uptake may be more effective in distributing iron to various cellular compartments than uptake of intact ferrichrome molecules with subsequent intracellular reduction. Shanzer and coworkers used fluorescence-labelled ferrichrome to analyze the entrance and distribution of ferrichrome in *Ustilago* and found that after intracellular reduction the desferri-ligand accumulates in the vacuole which prevents from intracellular reforming of the ferrichrome complex (Ardon et al. 1998). As observed earlier by Emery the desferri-ligand is excreted and can be used for another round of iron uptake. We now know that iron complexes can be reduced either intracelllarly or extracellularly depending on the fungal strain studied. An example is the extracelluar reductase in *S. cerevisiae*, first observed by Lesuisse et al. (1987) and later shown to occur also when ferrioxamines are reduced by an *Fre1* encoded gene (Dancis et al. 1992; Yun et al. 2001).

Extracellular and intracellular siderophores

Another aspect that has not often been discussed with siderophore production in fungi is the classification of extracellular and intracellular

siderophores. The distinction between intracellular and extracellular siderophores were already introduced by the group of Horowitz who showed that Penicillium strains contain different classes of siderophores which are partly intra and extracellular (Charlang et al. 1981). Early reports from our group showed that in *Neurospora crassa* two siderophores are produced, coprogen and ferricrocin. While coprogen is the major siderophore ecreted into the surrounding medium, ferricrocin remains largely inside the fungal cells, suggesting two different functions. Using Moessbauier spectroscopy we were able to assign ferricrocin a storage function in *N. crassa* and *A. ochraceous* (Matzanke et al. 1987). Moreover, intracellular ferricrocin is necessary during sporulation. When L-ornithine was omitted as a supplement in an ornithine-deficient *arg-5,ota,aga* mutant of *Neurospora crassa* sporulation was inhibited. The same conclusion was drawn from ferricrocin-negative mutants of *Aspergillus nidulans* (Haas 2003). Thus package of siderophores into spores is an essential process for spore production. We therefore believe that intracellular siderophores have at least two functions, storage and sporulation. The group of Horowitz had already shown that ferricrocin is also a germination factor for spores of *N. crassa* suggesting that ferricrocin stored into spores can finally be re-utilized during germination (Horowitz et al. 1976).

The transfer of iron inside the cell from an extracellular to an intracellular siderophore is kinetically feasible as Fe-hydroxamate complexes are all kinetically labile. Although in vitro exchange kinetics showed that the transfer is slow, the biological significans of a transfer of iron from exogenous coprogen to endogenous desferricrocin in *N. crassa* followed by Moessbauer spectroscopy was clearly shown (Matzanke et al. 1988). We recently found that *Schizosaccharomyces pombe* produces ferrichrome being mainly present intracellularly, but minor amounts could be detected in low-iron culture fluids (Schrettl et al. 2004a). This is surprising and points to a major intracellular role of ferrichrome in fission yeasts. Bakers yeast, *Saccharomyces cerevisiae*, has no siderophores at all, but still utilizes ferrichromes via a major facilitator transporter, Arn1p, which recognizes various ferrichrome-type siderophores (Heymann et al. 2000b). A similar transporter (Sid1p/Arn1p) mediates ferrichrome transport in in *Candida albicans*, which was proven by heterologous expression in the yeast *S. cerevisiae* (Heymann et al. 2002). In this investigation it was also shown that ferrichrome was required for epithelial invasion of *C. albicans* in a reconstituted human epithelium as a model of human oral mucosa, indicating that siderophores play an important role for pathogenic fungi. Although in this case the pathogen seems not to produce any siderophores, the growth promoting activity of siderophores could be clearly documented. It may be interesting to know if the transporter has other transport functions in *C. albicans.* Ferrichrome has also been detected in the human pathogenic dermatophytic fungi, *Microsporum* and *Trichophyton* (Mor et al. 1992) suggesting an iron sequestering activity during growth in epithelial layers.

The use of exogenous siderophores or xenosiderophores is an important aspect in microbiology, as some microorganisms do not produce siderophores but still utilize siderophores synthesized by other microorganisms. The feeding with siderophores is widely used in bioassays in order to demonstrate their growth promoting activity. Cross feeding has been observed with a variety of bacteria. In fungi utilization of bacterial siderophores has also been observed. Thus enterobactin, the predominant enterobacterial siderophore can be utilized by *Saccharomyces* which is a siderophore non-producer. A transporter for enterobactin (*ENB*1) in *S. cerevisiae* has been identified as a memberof the major facilitator superfamily (Heymann et al. 2000a), indicating that yeasts may profit from the presence of enterobacteria in low-iron habitats. The natural habitat of yeasts are sugar containing fruits and flowers of plants which are also colonized by a variety of bacteria which may support growth of the yeasts by excreting siderophores under iron limitation. The coexistence of bacteria and yeasts is seen in the milk product kefir being mixed cultures, which acidify milk and give it a pleasant flavour. A mutualistic relationship with regard to iron or siderophores may be a possible explanation as the habitat milk has a very low free iron content due to iron binding to lactoferrin. Despite

of its low iron availability in milk, cheese is often a preferred substrate for fungi. Thus *P. camemberti* and *P. roquefortii* are well known fungi for cheese production. Although a variety of Penicillium strains had been studied earlier for siderophore production, resulting in ferrichrome and coprogen production (Zähner et al. 1963), there was also a report on the extraction and identification of ferrichrome and coprogen fom blue cheese (Ong and Neilands 1979) which confirmed the capability of these fungi to grow in cheese by producing siderophores in order to extract iron from milk constituents.

Transport systems

Although the biosynthesis of siderophores has been studied in fungi to some extent by several groups (Wang et al. 1989; Mei et al. 1993; Haas 2003; Eisendle et al. 2003), the analysis of siderophore transport systems has been the main focus of many laboratories (Leong and Winkelmann 1998). Due to the fact that the whole genome of *Saccharomyces cerevisiae* is known, containing about 6000 genes, and gene disruption techniques and mutant strain collections are available, the mineral nutrient and trace element homeostasis has been characterized as the yeast ionome (Eide et al. 2005). Based on the knowledge of the genome of *S. cerevisiae*, we have identified three siderophore transport genes (*TAF1*, *ENB1* and *ARN1*) as members of the major facilitator family by deletion of the transporter genes (Heymann et al. 1999, 2000a, b). These siderophore transporters show substrate specificity in terms of recognition of different siderophore classes and as facilitators depend on a proton gradient. The siderophore transporter genes, designated *ARN1–4*, were also identified through cDNA microarrays and were found to be regulated by the major iron-dependent AFT1 transcription factor (Yun et al. 2000). Although *S. cerevisiae* is unable to synthesize its own siderophores, it turned out to be a general tool for the study of fungal siderophore transport. Thus, heterologous expression of transport proteins in disrupted *S. cerevisiae* mutant strains allowed to study the function of transport proteins from other fungi like *Schizosaccharomyces pombe* (Pelletier et al. 2003) or *Candida albicans* (Hu et al. 2002; Heymann et al. 2002). Even cell wall material is actively involved in iron transport in fungi, as shown by the recently detected cell wall mannoproteins (Fit proteins, facilitators of iron transport) in *S. cerevisiae* (Protchenko et al. 2001). In mycelial fungi of the Ascomycetes and Basidiomycetes certain biosynthetic genes have been identified of which the L-ornithine-N^5–monooxygenase is the first committed step in the biosynthesis of hydroxamate siderophores (Mei et al. 1993). Major contributions to the molecular genetics of siderophore in filamentous fungi have recently been made by the group of Haas (2003) on *Aspergillus nidulans* and *A. fumigatus* strains respectively (Schrettl et al. 2004b).

Regulation

Iron acquisition via siderophores means a considerable investment of biosynthetic energy. Moreover, as siderophores are made for external iron sequestering purposes, many of the siderophores get lost during excretion and only few will return as iron-loaded molecules to support growth of the producing microorganism. Therefore, the production and excretion of siderophores is regulated by regulatory proteins that respond to the availability of external iron. In many gram-negative and gram-positive bacteria ferric uptake regulation proteins (Fur) are present. Since the first observation of a constitutive siderophore mutant in Salmonella, named *fur* (Ernst et al. 1978), the *fur* gene has been cloned (Hantke 1984) and the transcriptional repression by iron in fur+ strains has been shown to control a large number of genes in iron acquisition systems (de Lorenzo et al. 2004). The model of repression requires binding of internal ferrous iron to the Fur protein, which then binds to the target DNA (Fur Box) and inhibits RNA polymerase access to the promoters of iron-regulated genes. Under low ferrous iron conditions the Fur protein dissociates from the Fur box and the genes for biosynthesis of siderophores and their transport systems are activated. In high G + C gram-positive bacteria like *Corynebacterium*, *Mycobacterium* and *Streptomyces* a protein named DtxR (diphteria toxin

regulator) is prevailing which acts in a similar way as the Fur protein.

The corresponding transcriptional repressor proteins in fungi are the GATA factor proteins (Scazzocchio 2000), which contain the GATA type zinc fingers that bind to promoters of siderophore biosynthetic genes (An et al. 1997). So far URBS1 in *Ustilago maydis* (Voisard et al. 1993; An et al. 1997), SRE in *N. crassa* (Zhou et al. 1998), SREP in *P. chrysogenum* (Haas et al. 1997), SREA in *A. nidulans* (Haas et al. 1999; Oberegger et al. 2001) and GAF2p in *Schizosaccharomyces pombe* (Hoe et al.1996; Pelletier et al. 2003) have been identified as GATA factors which negatively regulate the biosynthesis and transport of siderophores in most fungi. However, in *S. cerevisiae* and *C. albicans* the transcription factor Aft1 binds to the promotors of these genes and positively activates gene expression in the absence of iron. (Yamaguchi-Iwai et al. 1995, 1996) Thus, sensing of the external iron concentration is needed to regulate intracellular biosynthesis of siderophores and transport proteins in bacteria and fungi. Therefore, regulation of siderophore production may be regarded as one of the most important cellular function in siderophore ecology. Although the impact of siderophore regulation has been shown genetically by introduction of various genes into disrupted strains (*C. cerevisiae* fet3Δ, arn1–4Δ) the ecology behind these regulons have not been emphasized. Sensing of iron in a natural environment means surviving and helps outcompeting other microorganisms that are unable to adapt their iron metabolism. Thus, every microorganism that has a highly sensitive iron regulation will be superior to other colonizing species. That means ecological niches having a low iron content will be first colonized by aerobic microorganisms that can rapidly up-regulate genes for the biosynthesis of siderophores and their cognate transport systems. The question is, however, where are the low iron habitats in natural ecosystems? The marine ecosystem is well known for its low iron content, especially the open oceans where iron leveln in surface water range from 0.02–1 nM (Butler 2005) In addition many fresh water lakes and calcerous soils possess a low iron content which may inhibit bacterial and planctonic as well as plant growth. The most strict low iron conditions prevail in animals and humans, where free iron is virtually absent and any iron is bound to host proteins like ferritin, transferrin or lactoferrin. Only certain pathogens can multiply in this low iron environment by sequestering iron directly from the host proteins by the use of transferrin binding proteins (Tbp) and ferric binding proteins (Fbp A and B) in the periplasm-to-cytosol transport as found in *Neisseria* (Cornelissen and Sparling 2004). Cell lysis by toxins and degradation of proteins by proteases with subsequent iron scavenging by excretion of siderophores is also a method of iron acquisition in *Pseudomonas aeruginosa*. There are many ways by which pathogens can utilize iron from their hosts, and also heme uptake is wide-spread, but the most perfect system seems to be the siderophore system.

A further important aspect of siderophore ecology is energy saving. The biosynthesis of siderophores needs energy in form of carbon sources and ATP. Although this sounds trivial, energy and growth rates determine the kind of population that will colonize a low-iron habitat. Microorganisms that continuously produce siderophores are unknown in nature but can be constructed in the laboratory as for example *fur* mutant bacteria. A fur mutant may not survive for longer times in ecosystems containing a low nutrient content and is probably outcompeted by bacteria that can down-regulate siderophore biosynthesis and rather invest energy for metabolism and growth. The same is true for regulating siderophore biosynthesis in fungi. Siderophore production in fungi starts just after germination from conidiospores. Most conidiospores still contain a certain amount of siderophores packed into the spore wall material, which is released during germination (Matzanke et al. 1988). In previous studies Horowitz et al. coined the name germination factor for ferricrocin (Horowitz et al. 1976) at a time where ferricrocin was already known as a siderophore in *Neurospora crassa* (Winkelmann and Zähner 1973; Winkelmann 1974). Indeed sporulation cannot proceed in strains where genes of siderophores are knocked out (Eisendle et al. 2003).

Siderophores and pH

An important ecological factor for siderophore biosynthesis is the external pH. In *A. nidulans* pH regulation of gene expression is mediated by the wide-domain zinc finger transcription factor PacC, which controls biosynthesis and uptake of siderophores (Eisendle et al. 2004). Using acidity- and alkalinity-mimicking mutants is was shown that siderophore production was down-regulated at low pH and up-regulated at high pH. Siderophore production of the wild type increased 35.7-fold as the culture was raised from 4.2 to 7.0. Thus, activated PacC acts as both, an activator of alkaline expressed genes and a repressor of acid-expressed genes. The rational behind this regulation may be that under alkaline conditions it is more difficult to aquire insoluble iron whereas at acid pH ferric iron is to a certain extent soluble (~1 μM at pH3). However, as is well known fungi excrete a variety of organic acids that acidify the surrounding of the mycelia. We have shown earlier that iron deficiency enhance acid production (succinate, malate and citrate) by comparing wild type *N. crassa* 74A with with an ornithine-deficient mutant (*arg-5, ota, aga*) which is unable to sythesize hydroxamate siderophores (Winkelmann 1979). Siderophores and organic acids are both suitable as iron oxide dissolution agents (Kraemer 2004), which in combination increase the amount of iron available from the environment. The presence of acid anions may also catalyze interligand iron exchange between different siderophores or between siderophores and other chelating compounds involving a ternary complex as a transition state (Monzyk and Crumbliss 1983; Mies et al. 2006). Most ferric hydroxamates are stable down to pH 1 without loosing the complexed iron atom and a simultaneous excretion of both, acids and siderophores seems advantageous. This has recently been confirmed by studies on oxalate and ferricrocin exudation by the extramatrical mycelium of an ectomycorrhizal fungus, *Hebeloma crustuliniforme*, in symbiosis with *Pinus sylvestris* (van Hees et al. 2006). Both, oxalate (μM range) and ferricrocin (nM range) act synergistically as iron dissolution agents. However, although their role in iron supply for the fungus is obvious, the role in iron nutrition of the plant root remains unsolved.

We have recently reported on an analogous pH-dependence of siderophore biosynthesis in an *E. coli* strain Nissle 1917, where environmental factors influence the production of enterobactin, salmochelin, aerobactin, and yersiniabactin (Valdebenito et al. 2006). Yersiniabactin and salmochelin were maximally produced under neutral to alkaline conditions, whereas aerobactin was maximally produced at a more acidic pH (pH 5.6). Although *E. coli* Nissle 1917 is a non-pathogenic organism, related uropathogenic *E. coli* strains (UPEC) are known for the production of essentially the same collection of siderophores (Valdebenito et al. 2005). As an explanation we think that uropathogenic *E. coli* strains may need to adapt to the pH of the urine, which varies between pH 4.6 and 8.0. This further underlines the significance of the biosynthesis of a set of different siderophores in one microorganism.

References

An Z, Mei B, Yuan WM, Leong SA (1997) The distal GATA sequences of the *sid1* promotor of Ustilago maydis mediate iron repression of siderophore production and interact directly with Urbs1, a GATA family transcription factor. Embo J 16:1742–1750

Anke H, Kinn J, Bergquist K-E, Sterner O (1992) Production of siderophores by strains of the genus *Trichoderma*. BioMetals 4:176–180

Ardon O, Nudelman R, Caris C, Libman J, Shanzer A, Chen Y, Hadar Y (1998) Iron uptake in *Ustilago maydis*: tracking the iron path. J Bacteriol 180:2021–2026

Bar-Ness E, Chen Y, Hadar Y, Marschner H, Römheld V (1991) Siderophores of *Pseudomonas putida* as an iron source for dicot and monocot plants. Plant Soil 130:231–241

Bar-Ness E, Hadar Y, Chen Y, Römheld V, Marschner H (1992) Short-term effects of rhizosphere microorganisms on Fe uptake from microbial siderophores by maize and oat. Plant Physiol 100:451–456

Brüggemann W, Maas-Kantel K, Mog PR (1993) Iron uptake by leaf mesophyll cell: The role of the plasma membrane-bound ferric chelate reductase. Planta 190:151–155

Butler A (2005) Marine siderophores and microbial iron metabolism. BioMetals 18:369–374

Charlang G, Bradford NG, Horowitz NH, Horowitz RM (1981) Cellular and extracellular siderophores of *Aspergillus nidulans* and *Penicillium chrysogenum*. Mol Cell Biol 1:94–100

Cline GR, Reid CPP, Powell P, Szaniszlo PJ (1984) Effects of a hydroxamate siderophore on iron absorption by Sunflower and Sorghum. Plant Physiol 76:36–39

Cornelissen CN, Sparling PF (2004) Neisseria. In: Crosa JH, Mei AR, Payne SM, (eds) Iron Transport in Bacteria. ASM Press, Washington DC, pp 256–272

Crosa JH, Mey AR, Payne SM (eds) (2004) Iron transport in bacteria. ASM Press Washington DC

Dancis A, Roman DG, Anderson GJ, Hinnebusch AG, Klausner RD (1992) Ferric reductase of *Saccharomyces cerevisiae*: Molecular characterization, role in iron uptake, and transcriptional control by iron. Proc Natl Acad Sci (USA) 89:3869–3873

De Lorenzo V, Martin JP, Escolar L, Pesole G, Bertoni G (2004) Mode of binding of the Fur protein to target DNA: Negative regulation of iron-controled gene expression. In: Crosa JH, Mei AR, Payne SM (eds) Iron Transport in Bacteria. ASM Press, Washington DC, pp 185–196

Diekmann H, Krezdorn E (1975) Stoffwechselprodukte von Mikroorganismen. 150 Mitteilung. Ferricrocin, Triacetylfusigen und andere Sideramine aus Pilzen der Gattung Aspergillus, Gruppe Fumigatus. Arch Microbiol 106:191–194

Drechsel H, Winkelmann G (1997) Iron chelation and siderophores. In: Winkelmann G, Carrano CJ (eds), Transition Metals in Microbial Metabolism. Harwood Acadmic Publishers, Amsterdam, pp 1–49

Ecker DJ, Lancaster JR, Emery T (1982) Siderophore iron transport followed by electron paramagnetic resonance spectroscopy. J Biol Chem 257:8623–8626

Eide DJ, Clark S, Nair TM, Gehl M, Gribskov M, Guerinot ML, Harper JF (2005) Characterization of the yeast ionome: a genome-wide analysis of nutrient mineral and trace element homeostasis in Saccharmyces cerevisiae. *Genome Biology* 6, R77 Open Access

Eisendle M, Oberegger H, Zadra I, Haas H (2003) The siderophore system is essential for viability of *Aspergillus* nidulans: functional analysis of two genes encoding L-ornithine-N^5-monooxigenase *(SidA)* and a non-ribosomal peptide synthetase *(sidC)*. Mol Microbiol 49:359–375

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

Emery T (1987) Reductive mechanisms of iron assimilation. In Winkelmann G, van der Helm D, Neilands JB (eds), Iron Transport in Microbes, Plants and Animals. VCH Verlagsgesellschaft, Weinheim, pp 235–250

Ernst JF, Bennet RL, Rothfield LI (1978) Cunstitutive expression if the iron-enterochelin and ferrichrome uptake systems in a mutant strain of *Salmonella typhimurium*. J Bacteriol 135:928–934

Essén SA, Bylund D, Holmström SJM, Moberg M, Lundström US (2006) Quantification of hydroxamate siderophores in soil solutions of podzolic soil profiles in Sweden. BioMetals 19:269–282

Fernandéz V, Winkelmann G, Ebert G (2004) Iron supply to tabacco plants through foliar application of iron citrate and ferric dimerum acid. Physiologia Plantarum 122:380–385

Fernandéz V, Ebert G, Winkelmann G 2005. The use of microbial siderophores for foliar iron application studies. Plant Soil 272:245–252

Haas H, Angermayr K, Stoffler G (1997) Molecular analyis of a *Penicillium chrysogenum* GATA factor encoding gene *(sreP)* exhibiting significant homology to the *Ustilago maydis urbs1* gene. Gene 184:33–37

Haas H, Zadra I, Stöffler G, Angermeyer K (1999) The *Aspergillus nidulans* GATA factor SREA is involved in regulation of siderophore biosynthesis and control of iron uptake. J Biol Chem 274:4613–4619

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 (1984) Cloning of the repressor protein gene of iron regulated systems in *E. coli* K12. Mol Gen Genet 197:337–341

Haselwandter K, Dobernigg B, Beck W, Jung G, Cansier A, Winkelmann G (1992) Isolation and identification of hydroxamate siderophores of ericoid mycorrhizal fungi. BioMetals 5:51–56

Haselwandter K, Winkelmann G (1998) Identification and characterization of siderophores of mycorrhizal fungi. In: Varma A (ed) Mycorrhiza Manual. Berlin, Springer-Verlag, pp 243–254

Haselwandter K, Winkelmann G (2002) Ferricrocin—an ectomycorrhizal siderophore of *Cenococcum geophilum*. BioMetals 15:73–77

Haselwandter K, Passler V, Reiter S, Schmid DG, Nicholson G, Hentschel P, Albert K, Winkelmann G (2006) Basidiochrome—a novel siderophore of the orchidaceous mycorrhizal fungi *Ceratobasidium* and *Rhizoctonia* spp. BioMetals 19:335–343

Hesseltine CW, Pidacks C, Whitehill AR, Bohonos N, Hutchings BL, Williams JH (1952) Coprogen, e new groth factor for coprophilic fungi. J Am Chem Soc 74:1362–1363

Heymann P, Ernst JF, Winkelmann G.(1999) Identification of a fungal triacetylfusarinine C siderophore transport gene (*TAF1*) in *Saccharomyces cerevisiae* as a member of the major facilitator superfamily. BioMetals 12:301–306

Heymann P, Ernst JF, Winkelmann G (2000a) A gene of the major facilitator superfamily encodes a transporter for enterobactin (Enb1p) in *Saccharomyces cerevisiae*. BioMetals 13:65–72

Heymann P, Ernst JF, Winkelmann G (2000b) Identification and substrate specificity of a ferrichrome-type siderophore transporter (Arn1p) in *Saccharomyces cerevisiae*. FEMS Microbiol Lett 186:221–227

Heymann P, Gerads M, Schaller M, Dromer F, Winkelmann G, Ernst J (2002) The siderophore iron transporter of *Candida albicans* (Sit1p/Arn1p) mediates uptake of ferrichrome-type siderophores and is required for epithelical invasion. Infect Immun 70:5246–5255

Hoe KL, Won MS, Yoo OJ, Yoo HS (1996) Molecular cloning of GAF2, a *Schizosaccharomyces pombe* GATA factor, which has two zinc-finger sequences. Biochem Mol Biol Int 39:127–135

Hördt W, Römheld V, Winkelmann G (2000) Fusarinines and dimerum acid, mono- and dihydroxamate siderophores from *Penicillium chrysogenum*, improve iron utilisation by strategy I and strategy II plants. BioMetals 13:37–46

Horowitz NH, Charlang G, Horn G, Williams NP (1976) Isolation and identification of the conidial germination factor of *Neurospora crassa*. J Bacteriol 127:135–140

Hu CJ, Bai C, Zheng XD, Wang YM (2002) Characterization and functional analysis of the siderophore-fe transporter CaArn1p in *Candida albicans*. J Biol Chem 11:11

Jalal MAF, van der Helm D (1981) Isolation and spectroscopic identification of fungal siderophores. In: Winkelmann G (ed), Handbook of Microbial Iron Chelates. Bocca Raton, CRC Press, pp 235–269

Jalal MAF, Morcharla R, Barnes CL, Hossain MB, Powell DR, Eng-Wilmot DL, Grayson SL, Benson BA, van der Helm D (1984) Extracellular siderophores from *Aspergillus ochreaceous*. J Bacteriol 158:683–688

Jalal MAF, Love SK, van der Helm (1986) Siderophore-mediated iron(III) uptake in *Gliocladium virens*, 1. Properties of cis-fusarinine, trans-fusarinine, dimerum acid, and their ferric complexes. J Inorg Chem 28:417–430

Kraemer SM (2004) Iron oxide dissolution and solubility in the presence of siderophores. Aquat Sci 66:3–18

Konetschny-Rapp S, Jung G, Huschka H.-G, Winkelmann G (1988) Isolation and identification of the principal siderophore of the plant pathogenic fungus Botrytis cinerea. BioMetals 1:90–98

Lesuisse E, Raguzzi F, Crichton RR (1987) Iron uptake by the yeast *Saccharomyces cerevisiae*: involvement of a reduction step. J Gen Microbiol 133:3229

Lesuisse E, Simon-Casteras M, Labbe P (1998) Siderophore-mediated iron uptake in *Saccharomyces cerevisiae* encodes a ferrioxamine B permease that belongs to the major facilitator superfamily. Microbiology 144:3455–3462

Leong SA, Winkelmann G (1998) Molecular biology of iron transport in fungi. Met Ions Biol Syst 35:147–186

Mac Arthur JV (ed) (2006) Microbial ecology. An evolutionary approach. Elsevier, Academic Press Publ. Amsterdam, New York

Matzanke BF, Bill E, Trautwein AX, Winkelmann G (1987) Role of siderophores in iron storage in spores of *Neurospora crassa* and *Aspergillus ochraceus*. J Bacteriol 169:5873–5876

Matzanke BF, Bill E, Trautwein AX, Winkelmann G (1988) Ferricrocin functions as the main intracellular iron-storage compound in mycelia of *Neurospora crassa*. BioMetals 1:18–25

Mei B, Budde AD, Leong SA (1993) *sid1*, a gene initiating siderophore biosynthesis in *Ustilago maydis*: Molecular characterization, regulation by iron, and role in phytopathogenicity. Proc Natl Acad Sci USA 90:903–907

Mies KA, Wirgau JL, Crumbliss AL (2006) Ternary complex formation facilitates a redoxmechanism for iron release from a siderophore. BioMetals 19:115–126

Monzyk B, Crumbliss AL (1983) Factors that influence siderophore-mediated iron bioavailability: Catalysis of interligand iron(III) transfer from ferrioxamine B to EDTA by hydroxamic acids. I Inorg Biochem 19:19–39

Mor H, Kashman Y, Winkelmann G, Barash I (1992) Characterization of siderophores produced by different species of the dermatophytic fungi *Microsporum* and *Trichophyton*. BioMetals 5:213–216

Neilands JB (1952) A crystalline organo iron compound from a rust fungus *Ustilago spaerogena* . J Am Chem Soc 74:4846–4847

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

Ong SA, Neilands JB (1979) Siderophores in microbially processed cheese. J Agr Food Chem 27:990–995

Pelletier B, Beaudoin J, Philpott CC, Labbé S (2003) Fep1 represses expression of the fission yeast Schizosaccharomyces pombe siderophore-iron transport system. Nucleic Acids Res 31:4332–4344

Powell PE, Cline GR, Reid CPP, Szaniszlo PJ (1980) Occurrence of hydroxamate siderophore iron in soils. Nature 287:833–834

Powell PE, Szaniszlo PJ, Cline GR, Reid CPP (1982) Hydroxamate siderophores in the iron nutrition of plants. J Plant Nutr 5:653–673

Prabhu V, Biolchini PF, Boyer GL (1996) Detection and identification of ferricrocin produced by ectendomycorrhizal fungi in the genus *Wilcoxina*. BioMetals 9:229–234

Protchenko O, Ferea T, Rashford J, Tiedeman J, Brown PO, Botstein D, Philpott CC (2001) Three cell wall mannoproteins facilitate the uptake of iron in *Saccharomyces cerevisiae*. J Biol Chem 276:49244–49250

Scazzocchio C (2000) The fungal GATA factors. Curr Opin Microbiol 3:126–131

Schrettl M, Winkelmann G, Haas H (2004a) Ferrichrome in *Schizosaccharomyces pombe* – an iron transport and iron storage compound. BioMetals 17:647–654

Schrettl M, Bignell E, Kragl C, Joechl C, Rogers T, Arst HN Jr., Haynes K, Haas H (2004b) Siderophore biosynthesis but not reductive iron assimilation is essential for *Aspergillus fumigatus* virulence. J Exp Med 200:1213–1219

Szaniszlo PJ, Powell PE, Reid CPP, Cline GR (1981) Production of hydroxamate siderophore iron chelators by ectomycorrhizal fungi. Mycologia 73:1158–1174

Valdebenito M, Bister B, Reissbrodt R, Hantke K, Winkelmann G (2005) The detecction of salmochelin and yersiniabactin in uropathogenic *Escherichia coli* strains by a novel hydrolysis–fluorescens-detection method. Int J Med Microbiol 295:99–107

Valdebenito M, Crumbliss AL, Winkelmann G, Hantke K (2006) Environmental factors influence the production of enterobactin, salmochelin, aerobactin, and

yersiniabactin in *Escherichia coli* strain Nissle. Int J Med Microbiol (in press)

van der Helm D, Baker JR, Eng-Wilmot DL, Hossain MB, Loghry RA (1980) Crystal structure of ferrichrome and a comparison with the structure of ferrichrome A. J Am Chem Soc 102:4224–4231

Van der Walt JP, Botha A, Eiker A (1990) Ferrichrome production by Lipomycetaceae. Syst Appl Microbiol 13:131–135

Van Hees PA, Rosling A, Essén S, Godbold DL, Jones DL, Finlay RD (2006) Oxalate and ferricrocin exudation by the extramatrical mycelium of an ectomycorrhizal fungus in symbiosis with *Pinus sylvestris*. New Phytologist 169:367–378

Villavicenio M, Neilands JB (1965) An inducible ferrichrome A-degrading peptidase from pseudomonas FC-1. Biochemistry 4:1092–1097

Voisard C, Wang J, McEvoy JL, Xu P, Leong SA (1993) *Urbs1*, a gene regulating siderophore biosynthesis in *Ustilago maydis*, encodes a protein similar to the erythroid transcription factor GATA-1. Mol Cell Biol 13:7091–7100

Wang J, Budde AD, Leong SA (1989) Analysis of ferrichrome biosynthesis in the phytopathogenic fungus *Ustilago maydis*: cloning of an ornithine-N^5-oxygenase gene. J Bacteriol 171:2811–2818

Warren RAJ, Neilands JB (1965) Mechanisms of microbial catabolism of ferrichrome A. J Biol Chem 240:2055–2058

Wiebe C, Winkelmann G (1975) Kinetic studies on the specificity of chelate-iron uptake in *Aspergillus*. J Bacteriol 123:837–842

Winkelmann G, Zähner H 1973. Stoffwechselprodukte von Mikroorganismen. 115. Mitteilung. Eisenaufnahme bei *Neurospora crassa* I. Zur Spezifität des Eisentransportes. Arch Mikrobiol 88:49–60

Winkelmann G (1974) Stoffwechselprodukte von Mikroorganismen. 132. Mitteilung. Uptake of iron by *Neurospora crassa*. III Iron transport studies with ferrichrome-type compounds. Arch Microbiol 98:39–50

Winkelmann G (1979) Surface iron polymers and hydroxy acids. A model of iron supply in sideramine-free fungi. Arch Microbiol 121:43–51

Winkelmann G (1986) Iron Complex Products. In: Rehm HJ, Reed G (eds) Biotechnology 4:215–243

Winkelmann G, Schmidtkunz K, Rainey FA (1996) Characterization of a novel Spirillum-like bacterium that degrades ferrioxamine-type siderophores. BioMetals 9:78–83

Winkelmann G, Busch B, Hartmann A, Kirchhof G, Süßmuth R, Jung G (1999) Degradation of desferrioxamines by Azospirillum irakense: Assignment of metabolites by HPLC/electrospray mass spectrometry. BioMetals 12:255–264

Winkelmann G, Winge DR (eds) 1994. Metal Ions in Fungi. Marcel Dekker, Inc. Ney York-Basel Hong Kong

Winkelmann G (2001) Siderophore transport in fungi. In: Winkelmann G (ed), Microbial Transport Systems. Weinheim, Wiley-VCH, pp 463–480

Winkelmann G (2002) Microbial siderophore-mediated transport. Biochem Soc Trans 30:691–696

Winkelmann G (2004) Ecology of Siderophores. In: Crosa JH, Mey AR, Payne SM (eds), Iron Transport in Bacteria. ASM Press, Washington DC, pp 437–450

Yamaguchi-Iwai Y, Dancis A, Klausner RD (1995) AFT1: a mediator of iron regulated transcriptional control in *Saccharomyces cerevisiae*. EMBO J 14:1231–1239

Yamaguchi-Iwai Y, Stearman R, Dancis A, Klausner RD (1996) Iron-regulated DNA binding by the AFT1 protein controls the iron regulon in yeast. EMBO J 15:3377–3384

Yehuda Z, Shenker M, Römheld V, Marschner H, Hadar Y, Chen Y (1996) The role of ligand exchange in the uptake of iron from microbial siderophore by gramineous plants. Plant Physiol 112:1273–1280

Yun CW, Tiedeman JS, Moore RE, Philpott CC (2000) Siderophore-iron uptake in *Saccharomyces cerevisiae*. J Biol Chem 275:16354–16359

Yun CW, Bauler M, Moore RE, Klebba PE, Philpott CC (2001) The role of the FRE family of plasma membrane reductases in the uptake of siderophore-iron in *Saccharomyces cerevisiae*. J Biol Chem 276:10218–10223

Zähner H, Keller-Schierlein W, Hütter R, Hess-Leisinger K, Deér A (1963) Stoffwechselprodukte von Mikroorganismen 40. Mitteilung. Sideramine aus Aspergillaceen. Arch Microbiol 45:119–135

Zhou LW, Haas H, Marzluf GA (1998) Isolation and characterization of a new gene, sre, which encodes a GATA-type regulatory protein that controls iron transport in *Neurospora crassa*. Mol Gen Genet 259:532–540