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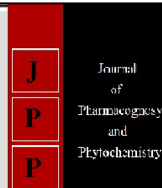

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Siderophore in fungal physiology and virulence

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Abstract
The iron plays free catalytic role in various vital cellular reactions and is not freely available in the environment due to host sequestration Maintaining the appropriate balance of iron between deficiency and toxicity requires fixed tuned-control system for iron uptake and storage. Most fungi express specific mechanism for acquisition of iron from the hosts they infect for their own survival. Siderophores, a low molecular weight iron chelator has the ability to form very stable and soluble complexes with iron. High affinity iron uptake systems such as siderophores mediated iron uptake and reductive iron assimilation (RIA) enable fungi to acquire limited iron from plant and animal host. Regulating the iron uptake is crucial to maintain iron homeostasis, a state necessary to avoid iron toxicity from iron abundance and simultaneously supply iron required to meet biochemical demand. Fungal cell used two different strategies to regulate iron acquisition that are activation during iron starvation and repression during iron deplete condition. Siderophores play diverse role in fungal host interaction, many of which have been delineated from gene deletion in NRPS, enzyme required for virulence, resistance to oxidative stresses, asexual/sexual development, iron storage and protection against iron induced toxicity in some fungal organism. It is demonstrated that fungal cell siderophores are virulence determinant. *Cochliobolus heterostrophus* NPS_6 deletion result in a strain that in reduced in virulence to corn and NPS6 deletion in *C. miyabeanus, F. gramineasum* and *A. brassiciola* also causes a reduction in pathogenicity to rice, wheat and Arabidopsis, respectively. Recently siderophores have been reported to play roles in plant-fungus symbioses.

Keywords: Siderophore**,** Fungal**,** Physiology**,** Virulence

Introduction
Siderophores are small, high-affinity iron chelating compounds secreted by grasses and microorganisms such as bacteria and fungi. Siderophores are amongst the strongest soluble Fe^{3+} binding agents known (Cornelis and Andrews, 2010).

The scarcity of soluble iron
Iron is essential for almost all life, essential for processes such as respiration and DNA synthesis. Despite being one of the most abundant elements in the earth's crust, the bioavailability of iron in many environments such as the soil or sea is limited by the very low solubility of the Fe^{3+} ion. This is the predominant state of iron in aqueous, non-acidic, oxygenated environments. It accumulates in common mineral phases such as iron oxides and hydroxide (the minerals that are responsible for red and yellow soil colours) hence cannot be readily utilized by organisms. Microbes release siderophores to scavange iron from these mineral phases by formation soluble Fe^{3+} complexes that can be taken up by active transport mechanisms. Many siderophores are nonribosomal peptides (NRPs), although several are biosynthesised independently (Challis, 2005).

Types of siderophores
Siderophores from tight complex with Fe^{3+} to overcome the problem of low bioavailability by solubilization. Siderophores are found in fungi, bacteria and plant, although mechanism by which this are produced differ in plants.
Siderophores can be classified into 3 groups depending on the chemical nature of moieties denoting the oxygen ligands for Fe^{3+}.
i) Catecholates
ii) Carboxylates
iii) Hydroxamate

With the exception of the carboxylate siderophore rhizoferrin which is produced by certain zygomycetes, all fungal siderophores identified so far are hydroxymates.

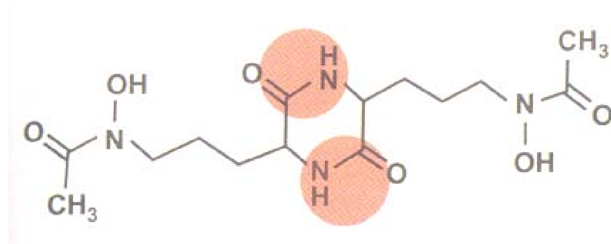
Fungal hydroxamates are derived from the non-proteinogenic amino acid ornithine and different acyl group and can be grouped into found structural families.

a) Rhodotorulic aicd
b) Fusarinines
c) Capprogens
d) Ferrichromes

a) Rhodotorulic acid

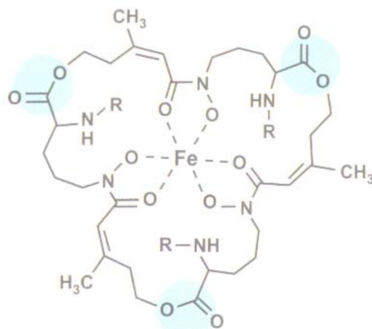
Rhodotorulic acid consist of two N^5- acetyl-N_5 hydroxyornithine units linked head to form diketopiperazine ring.

Rhodotorulic acid is produced by different species of basidiomycetous yeast genus *Rhodotorules*.



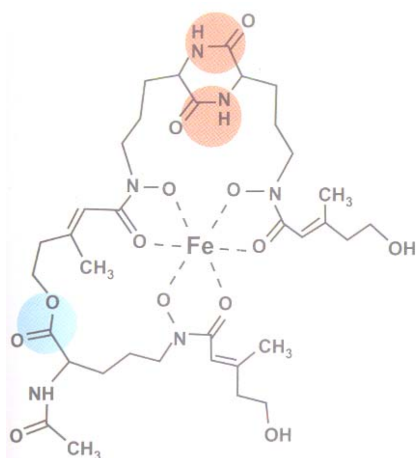
b) Fusarinines

The prototype of fusarinines, fusarinine C (or fusigen), consists of here N^5-*cis*-anhydromevalonyl N^5-hydroxyornithine residues (termed *cis*-fusarinine), linked by ester bonds in a head-to-tail fashion. Triacetylfusarinine C (TAFC) is formed by *N2*-acetylation of fusarinine C, which results in increased chemical stability and hydrophobicity.



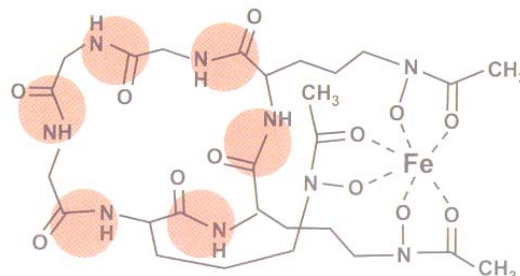
c) Capprogens

Caprogen are linear linear hydroxamates. Coprogen B contains two trans-fusarinine moieties connected head-to-head by a peptide bond to form a diketopiper-azine unit (dimerium acid) and a third trans-fusarinine molecule esterified to the C-terminal group of dimerium acid. N_2-acetylation and N_2-methylation of the latter fusarinine moiety allow for the diversity of the coprogen family. Moreover, one of the acyl groups of the diketopiperazine unit can be acetyl inserted of anhydromevalonyl. Fusarinines and coprogens are typically excerted by *Pezizomycota* species for iron acquisition.



d) Ferrichromes

Ferrichromes such as ferrichrome, ferrichrome A, and ferricrocin (FC) are cyclic hexapeptides consisting of three - N5-acyl-N5-hydroxyornithines and three amino acids (glycine, serine, or alanine). Acyl groups found in this family are acetyl, cis-, and trans-anhydromevalonyl, malonyl, and trans-β-methylglutaconyl. Ferrichromes are typically used for intracellular handling of iron by *Pezizomycota* species. *Taphrinomycota* and *Basidiomycota* also excrete ferrichromes.



Fungal iron acquisition

Control of iron uptake is considered the major fungus iron hemeostatic mechanisms because no excretory mechanism for iron has been identified yet.

In fungi four different mechanism for iron uptake has been characterized at molecular level.

a) Siderophore-mediated Fe^{3+} uptake
b) Reductive iron assimilation
c) Heme uptake
d) Direct iron uptake

a) Siderophore-mediated Fe^{3+} uptake

A fumigates excrete two types of siderophores fusirininc and TAFC to mobilise extra cellular iron. This ferric form of FC and TAFC are taken up by siderophores iron transporters. SIT constitute of subfamily of the major facilitator protein superfamily. SIT mediated iron uptake appears to be universally conserved in the fungal kingdom, even in species not producing siderophore. Possible reasons are the solubility and therefore high energy status of siderophore chelated iron in the putative role of stealing siderophores in microbial warfare. For intracellular release of iron, TAFC and FCS are hydrolysed partly by the esterase Estb.

b) Reductive iron assimilation

Reductive iron uptake start with reduction of ferric iron

source to the more soluble ferrous iron by plasma membrane –localized ferrireducatses. Subsequently the ferrous iron and re-onidized and imported by a protein complex consisting of the ferrioxidase Fetc and the iron permease FtrA.

c) Heme uptake

Different mechanisms for the direct use of host iron proteins such as transferring, lactroferrin, ferritin, and heme-proteins by binding to receptors on the cell surface, followed by extraction of the iron and import into the cytoplasm have been characterized in bacterial pathogens (173). In fungi, in contrast to bacteria, binding and uptake of only heme has been found. In *C. albicans,* the GPI-anchored cell surface mannoprotein Rbt5p is involved in heme uptake but the transport system is not yet known. Utilization of heme-iron requires intracellular degradation of heme by the endoplasmatic reticulum-localized heme oxygenase Hmxlp (181).

d) Direct Fe^{2+} uptake

At the molecular level, direct Fe^{2+} uptake has been studied exclusively in *S. cerevisiae*. Here, Fe^{2+} is taken up by Fet4p with an apparent K_m of approximately 30 μM. This low-affinity system is not specific for Fe^{2+} but also transports other metals, e.g., copper and zinc. Moreover, the NRAMP (Natural Resistance-Associated Macrophage Protein) family member Smf1p and fluid-phase endocytosis followed by mobilization of iron from the vacuole contributes to the iron supply of *S. cerevisiae*.

Siderophore biosynthesis

FsC is a cyclic tripcptic consisting of three N^5-anhydrimyvalonyl-N^5-hydroxornithinc residues linked by cster bonds, TAFC is the N^2-acctylated FsC, FC is a cyclic hexapeptide with the structure Gly-Ser-Gly-(N^5-acctyl-N^5-hydroxyornithinc)$_3$ and HFC is hydroxylated FC. The siderophore bios

FsC is a cyclic tripeptide consisting of three N^5-anhydromevalonyl-N^5-hydroxyornithine residues linked by ester bonds, TAFC is the N2-acetylated FsC, FC is a cyclic hexapeptide with the structure Gly-Ser-Gly-(N^5-acetyl-N5-hydroxyornithine)$_3$ and HFC is hydroxylated FC. The siderophore biosynthetic pathway characterized by reverse genetics is shown in Fig. 1. The first committed step in the biosynthesis of all four siderophores is hydroxylation of ornithine catalyzed by the ornithine monooxygenase SidA. Subsequently, the pathways for biosynthesis of extracellular and intracellular siderophores split owing to the transfer of different acyl-groups to N5-hydroxyornithine: an acetyl group is transferred via an unknown enzyme to form intracellular siderophores and anhydromevalonyl is transferred by transacylase SidF to form extracellular siderophores. Assembly of FsC and FC is catalyzed by two different non-ribosomal peptide synthetases (NRPS), SidD and SidC, respectively. TAFC and HFC are formed by SidG-mediated N2-acetylation of FsC and hydroxylation of FC, respectively. Additionally, the 40-phosphopantetheinyl transferase PptA is essential for siderophore biosynthesis because NRPS, along with polyketide synthetases and the lysine-biosynthetic a-aminoadipate reductase requires activation by this enzyme (Schrettl and Haas, 2011).

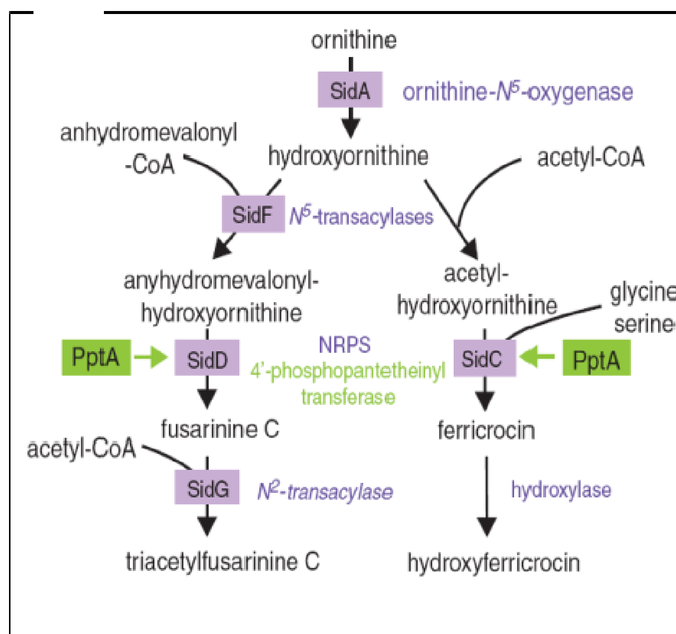


Fig. 1: Fungal siderophore biosynthesis pathway
[Source: Schrettl and Haas, 2011]

Biological functions of siderophores

Genetic elimination of extracellular siderophores (ΔsidF and ΔsidΔ mutants) decreases growth, conidiation and oxidative stress resistance under iron limited, but not iron sufficient conditions where other iron acquisition systems can compensate for the lack of siderophores. Elimination of intracellular siderophores (ΔsidC mutant) reduces conidiation and blocks sexual development (as shown in A. nidulans) owing to the role of FC in intracellular iron transport from substrate-contacting hyphae into aerial hyphae. The reduced intracellular iron supply causes conidial iron shortage, which impairs iron-dependent enzymes such as catalase A and consequently decreases conidial resistance to oxidative stress. Moreover, such conidia show delayed germination during iron starvation owing to the lack of conidial iron storage. Ablation of the entire siderophore system (ΔsidA mutant) combines the

defects caused by inactivation of either extracellular or intracellular siderophore biosynthesis and renders *A. fumigatus* extremely sensitive to iron starvation.

Both extracellular and intracellular siderophores contribute to pathogenic growth because elimination of the entire siderophore system (ΔsidA mutant) results in absolute avirulence of *A. fumigatus* in a murine model of invasive pulmonary aspergillosis, while deficiency in either extracellular (ΔsidF or ΔsidΔ mutants) or intracellular siderophores (ΔsidC mutants) causes partial attenuation of virulence. Blocking TAFC production, while concomitantly increasing FsC production (ΔsidG mutant) affects neither growth nor virulence, indicating that the structural differences between these two siderophores do not play a role in these processes. Consistent with a role in iron acquisition during infection, the *A. fumigatus* siderophores are able to remove iron from host proteins, such as transferring.

Genetic inactivation of RIA (ΔftrA mutant) does not affect virulence of *A. fumigatus*. Nevertheless, several lines of evidence indicate that RIA also plays a role during infection: (i) elimination of extracellular siderophores causes only partial attenuation of virulence, (ii) genome- wide expression profiling revealed induction of both the siderophore system and RIA during initiation of murine infection, and (iii) mutants lacking both RIA and the siderophore system (ΔftrAΔsidA double mutant) are unable to grow unless supplemented with siderophores or extremely high iron concentrations to fuel low-affinity iron uptake. Of note, RIA has been shown to be crucial for virulence of fungi that do not produce siderophores, such as C. albicans and C. neoformans.

Restoration of conidial HFC content by supplementation with FC during conidiation partially cures the virulence defect of ΔsidA conidia. This demonstrates a crucial role of the conidial siderophore during initiation of infection, most probably owing to its importance for germination and oxidative stress resistance.

Defects in the siderophore system decrease intracellular growth and survival of *A. fumigatus* after phagocytosis by murinc alveolar macrophages, which represent the first line of defense in the lung during pulmonary aspergillosis. Consistent with these findings, siderophore deficiency alters the immune response of immune macrophages against infection with *A. fumigatus*. Similarly, the siderophore system is also important for virulence of Histoplasma capsulatum, a dimorphic fungal pathogen that replicates in the yeast form within macrophages. Taken together, these data demonstrate that the siderophore system is crucial not only for extracellular but also for intracellular growth.

The evolutionary conserved role of iron in fungal virulence is underlined by the indispensable role of siderophores in various other experimental models of aspergillosis, that is, a murine cutaneous model, *Drosophila melanogaster*, and *Galleria mellonella*, as well as various phytopathogenic ascomycetes.

NRPs are large multifunctional enxymes that synthesize peptides from proteinogenic and nonproteinogenic precursors independently of the ribosome. Found exclusively in bacteria and fungi (plants use a different mechanism for siderophore biosynthesis), these enzymes are involved in the biosynthesis of a wide range of secondary metabolites. This thio-template-mechanism and the variety of possible substrates enormously expand the structural diversity of products. The involvement of NRPs in siderophore biosynthesis accounts for their categorization as secondary metabolites; our preference is to call this type of metabolite a “natural product” since iron up-take and storage is a primary metabolic function. NRPSs have a modular structure. On full module harbors all the catalytic units for incorporation of one amino acid (or amino acid-like) residues: an adenylation domain (A) for substrate specificity and activation, a peptidyl carrier domain (T) for attachment of the activated substrate, and a condensation domain (C) for bond formation. Linear NRPSs contain one module for every substrate to be incorporated into the peptide. In contrast, in iterative NRPs one or more modules are used repeatedly. Fungal siderophore NRPSs appear to belong to the latter class. The functionally characterized fungal NRPSs involved in siderophore biosynthesis are listed in Table-1.

Table 1: Characterized fungal siderophore NRPSs

NRPS name	Fungal species	Siderophores	Modular organization
Ferrichrome NRPSs			
Sid2	*U. maydis*	Ferrichrome	ATCATCATCTC
SidC	*A. nidulans*	FC	ATCATCATCTCTC
SidC	*A. fumigatus*	FC	ATCATCATCTCTC
Nps2	*C. heterostrophus*	FC	ATCATCATCATCTCTC
Nps2	F. graminearum	FC	ATCATCTCATCTCTC
SidFA/Fer3	*U. maydis*	Ferrichrome	ATCATCATCTCTC
Coprogen NRPSs			
Nps6	*A. Brassicicola*	N-dimethlcoprogen	ATCA*TTC
Nps6	*C. heterostrophus*	Coprogen, neocoprogen I, neocoprogen II	ATCA*TTC
Nps6	*C. miyabeanus*	nd	ATCA*TTC
Nps6	*N. crassa*	Coprogen	ATCA*TTC
Fusarinine NRPSs			
SidD	*A. fumigatus*	Fusarinine C	ATCA*TC
Nrps2	*E. festucae*	Novel fusarinine	np

[Source: Oide *et al*., 2007]

The identification and inactivation of siderophore transacylase-and NRPS-encoding genes allowed dissection of the role of intracellular and extracellular siderophores. In *Alternaria brassiciola, A. fumigatus, C. heterostrophus, Cochliobolus miyabeanus* and *F. graminearum,* loss of extracellular siderophores due to inactivation of the respective NRPSs (Table-1), which leaves the corresponding mutants absolutely dependent on RIA, the alternative high-affinity iron uptake system in these species, largely decreases the minimal inhibitory concentrations of Fe^{2+}- chelators such as membrane-impermeable bathophenanthroline disulfonate. During iron limitation, such mutants display a decrease in

growth rate, asexual sporulation, and oxidative stress resistance. Taken together, these data demonstrate that the alternative iron acquisition systems cannot fully compensate for the loss of siderophore-mediated iron uptake and consequently iron shortage. The increased sensitivity to oxidative stress might be explained by the dependence of several oxidative stress-detoxifying enzymes on iron, e.g., catalases and peroxidases require heme as cofactor. The phenotype of loss of intracellular siderophore is discussed below in the section siderophore-mediated iron storage.

Fungal siderophore uptake and utilization

In fungi, siderophore-iron chelates are usually taken up through transporters of the SIT subfamily of the major facilitator superfamily. These are secondary transporters with 12-14 predicted transmembrane domains, which likely function as proton symporters energized by the plasma membrane potential. As shown in transport studies in *N. crassa* and *S. cerevisiae*, recognition of siderophores is highly stereospecific, including that the binding to the transporter is not dependent on the overall size or hydrophobicity but on specific binding sites.

Fungal siderophore uptake has been studied in greatest detail in *S. cerevisiae*, which is not able to synthesize siderophores itself. This yeast expresses four different diserophore transporters that differ in substrate specificity (alternative gene names are indicated): Sitlp/Arn3p, Arn1p, Taflp/Arn2p, Enblp/Arn4p (84-86, 126, 219, 220). Notably, some variations in the specificity have been reported among different strains of *S. cerevisiae* (123). Two of these transporters are highly specific, Enblp/Arn4p for the bacterial catecholate siderophore enterobactin and Taflp/Arn2p for TAFC. The other two transporters show broad and overlapping specificity: Arn1p transports coprogen and a wide range of ferrichromes. Sit1p/Arn3p recognizes the bacterial hydroxamate ferrioxamine, B, coprogen, and a variety of ferrichromes laking anhydromevalonic acid (Fig. 2)

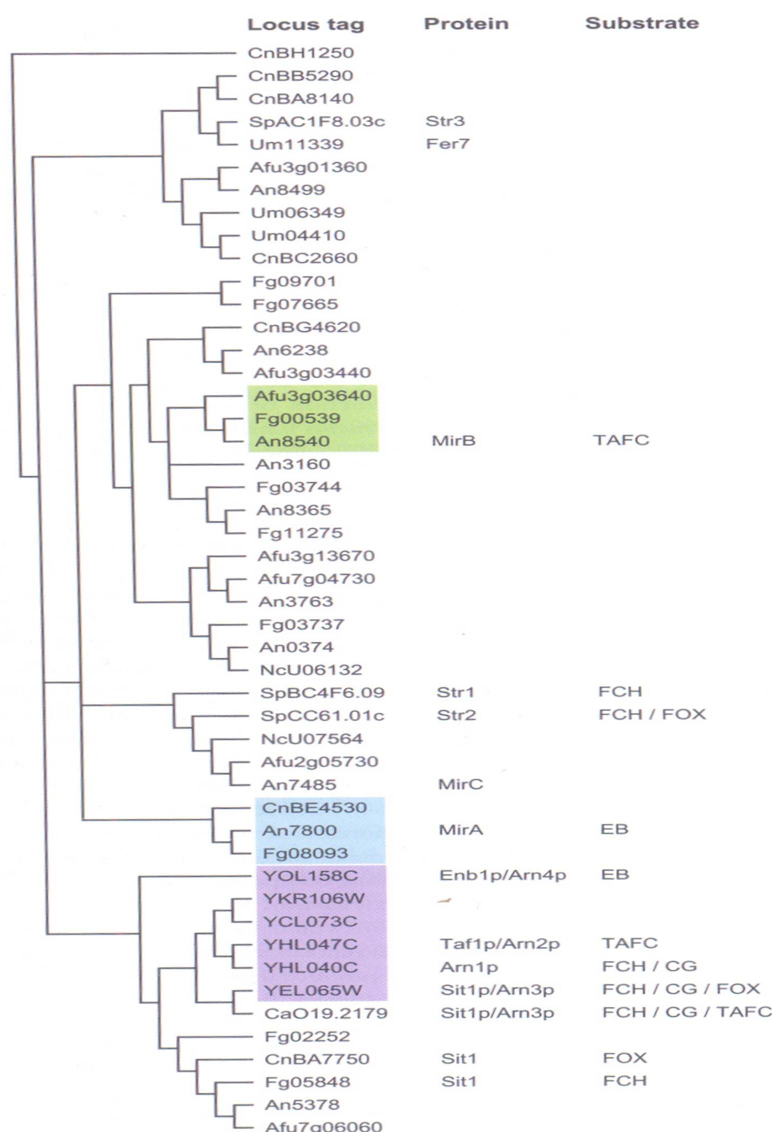


Fig. 2: Phylogenetic analysis of siderophore transporters from *S. cerevisiae* (Y), *C. albicans* (Ca), *A. nidulans* (An), *A. fumigatus* (Afu), *F. graminearum* (Fg), *C. neoformans* (Cn) and *U. maydis* (Um). Available gene names and substrate specificities are indicated. CG, coprogen; EB, enterobactin; FCH, ferrichromes, FOX, ferrioxamines; TAFC, triacetylfusarinine C. CnBH1250 is a major facilitator not belonging to the SIT-family and serves as an outgroup. *S. cerevisiae* transporters are boxed in *purple*. Permeases likely to be involved in transport of enterobactin and TAFC are boxed in *blue* and *green*, respectively.

[**Source:** Haas *et al*., 2008] [2]

Fungal iron storage
To ensure a steady of iron, cells need to store iron. In animals, plants and bacteria, iron is stored as ferritin, phytoferritin or bacterioferritin, respectively. With exception of zygomycetes, some of which produce a mycoferritin, ferritin-like molecules have not been discovered among fungi. Recently, purification of a ferritin-like protein of *Aspergillus parasiticus* has been reported, but the genomic sequence of this and other filamentous fungi do not appear to encode ferritin-like molecules. Two different mechanisms for iron storage have been described in fungi, vacuolar and siderophore-mediated iron storage.

Vacuolar iron storage
S. cerevisiae can grow for generations in the absence of exogenous iron, indicating a capacity to store iron intracellularly. Several studies have suggested the importance of the yeast vacuole as a facility for storage and detoxification of heavy metals; iron is probably stored in the ferric from as polyphosphate. Ccc1p mediates transport of iron into the vacuole. Export of iron from the vacuole is supported by the Smf1p paralog Smf3p and a complex consisting of Fet5p and Fth1p that is paralogous to the Fet3p-Ftr1p complex. The vacuolar membrane-localized metalloreductase Fre6p supplies Fe^{2+} to both vacuolar efflux systems. Consistent with roles in mobilization of vacuolar iron stores, deficiency in Smf3p or the Fe5p-Fth1p complex causes signs of iron starvation. In contrast, lack of Ccc1P causes sensitivity. In contrast, lack of Ccc1P causes sensitivity to iron. Identification of vacuolar membrane-localized orthologs to *S. cerevisiae* Fet5p and Fth1p in *F. graminearum* and of iron regulated Ccc1p orthologs in *A. nidulans* and *S. pombe* suggests vacuolar iron storage also in siderophore producing fungi. Several fungal species, including *A. nidulans* and *S. pombe*, do not possess homologs to *S. cerevisiae* Fet5p and Fth1p and therefore appear to lack this mode of vacuolar iron mobilization.

Siderophore mediated iron storage
All siderophore producing fungi studied to date posses intracellular siderophores as iron storage molecules. *U. sphaerogena* and *U. maydis* excrete ferrichrome and ferrichrome A, but only ferrichrome serves as an iron storage compound and accounts for 50 per cent of the cellular iron pool. The *Pexixomycota* investigated so far contain FC as intracellular siderophore in hyphae and condia. In *A. fumigatus*, however, FC was found to be hydroxylated for storage in conidia. FC, or its derivative, constitutes 47 to 74 per cent of the total conidial iron content in *A. nidulans, A. fumigatus, Aspergillus ochraceus* and *N. crassa*. Consistently, inactivation of FC biosynthesis reduces the iron content of conidia by 34 and 76 per cent in *A. nidulans* and *A. fumigatus*, respectively. Moreover, in both *Aspergillus* species, loss of FC biosynthesis decreases resistance to hydrogen peroxide of conidia due to a decrease in the non-dependent catalase. A activity as shown in *A. fumigatus*. These findings suggest a role of FC in iron supply during development. In agreement, a lack of FC reduces the rate of asexual apoulation in *A. nidulans* and *A. fumigatus,* but not in *C. heterostrophus* and it affects sexual development in *A. nidulans, C. heterostrophus* and *G. zeae*.

Regulation of iron homeostasis and its role in virulence
In *A. fumigatus*, iron starvation causes extensive transcriptional remodeling that is mediated by the two central transcription factors, SreA and HapX (Fig. 2) (Schrettl and Haas, 2011).

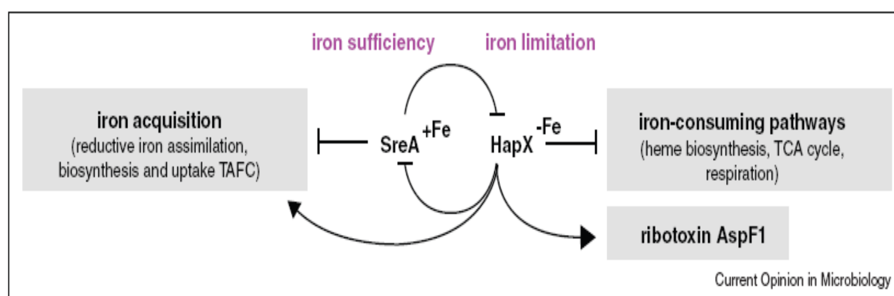


Fig 3: Iron regulation in *A. fumigatus*
[Source: Schrettl and Haas, 2011]

The DNA-binding GATA-factor SreA represses RIA and the siderophore system during iron sufficiency in order to avoid toxic effects. The bZip-transcription factor HapX physically interacts with the DNA-binding CCAAT-binding complex (as shown in *A. nidulans*) and represses iron-consuming pathways such as heme biosynthesis, respiration and ribosome biogenesis during iron starvation to spare iron]. Furthermore, HapX activates synthesis of the ribotoxin AspF1 and siderophores, the latter partly by ensuring a supply of the precursor, ornithine. SreA and HapX are interconnected in a negative feedback loop: SreA represses expression of hapX during iron sufficiency, while HapX represses sreA during iron starvation. In *A. nidulans*, inactivation of both regulators is synthetically lethal underlining the importance of iron metabolism for cellular survival. In agreement with the expression pattern and mode of action, detrimental effects of inactivation of SreA or HapX are confined to growth during iron sufficiency or starvation, respectively.
Deficiency in HapX, but not SreA, attenuates virulence of *A. fumigatus* in a murine model of aspergillosis, which underlines the crucial role of adaptation to iron limitation in virulence. Most fungal species possess orthologs of SreA and HapX, and the crucial role of HapX orthologs in virulence has been demonstrated in *C. albicans* and *C. neoformans*. A notable exception is the prototypical yeast S. cerevisiae, which employs entirely different regulators. Owing to the central metabolic role of iron, iron regulation is interconnected with various other regulatory circuits in Aspergilli, for example, pH regulation, gluconeogenesis, zinc metabolism, and oxidative stress response. Recently, deficiency in the transcription factor AcuM was shown to impair both gluconeogenesis and high-affinity iron uptake in *A. fumigatus*, whereby only the latter was suggested to be responsible for the virulence defect caused by AcuM-

deficiency. Various other metabolic links are expected to be identified in the future.

Siderophores and interaction with the host
Animal-pathogenic fungi
The microbial quest for iron in mammalian hosts is crucial for successful pathogenesis as, in this environment, iron is tightly bound by carrier proteins such as transferring, ferritin, and lactoferrin, leaving free iron concentrations in-sufficient for sustained microbial growth. A pathogen's requirement for iron is the basis for an elaborate mammalian defense system against microbial infection, which relies upon iron-withholding mechanisms to deny access to iron for invading microorganisms. Upon inflammation, iron is withheld by macrophages, leaving extracellular fluids iron-depleted, causing so-called anemaia of chromic disease. The crucial role of siderophores in virulence is reflected by the fact that mammals possess at least two proteins, the lipocalins Lcn2 and Lcn1, that are able to sequester siderophores. Lcn2, also called siderocalin or NGAL, protects mammals from infection with *E. coli* by sequestering the siderophore enterobactin and preventing iron acquisition. However, glycosylation of enterobactin inhibits recognition by Lcn2 and allows evasion of the native immune system, which represents and excellent example of host-pathogen coevolution. Lcn2 binds its substrates with high affinity (K_D of about 0.5 μM) but has a rather narrow substrate specificity by recognizing only particular catecholate but not hydroxamate siderophores. Lcn1 has a broader substrate specificity by recognizing a varietiy of bacterial and fungal catecholate and hydroxamate siderophores and therefore has bacteriostatic and fungistatic activity, but binds its substrates with lower affinity (K_D of about 0.2 μM). Various bacteria produce extracellular siderophores, and in many cases their involvement in virulence against animals has been demonstrated. In contrast to most fungi, bacteria do not employ intracellular siderophores but use ferritin and bacteriogferritin for iron storage. Recent studies have determined that the ability of *A. fumigatus* to acquire iron and survive in serum involves siderophore-mediated removal of iron from host transferring, implying a role for siderophore biosynthesis *in vivo*. Consistently, siderophore-deficient *A. fumigatus* mutants are not able to grow on blood agar plates. Abrogation of *A. fumigatus* siderophore biosynthesis by inactivation of *sidA* (Fig. 1) prevents initiation of mammalian infection using a mouse model for pulmonary aspergillosis. In contrast, inactivation of *A. fumigatus* RIA by deletion of the iron premease-encoding *ftr*A is inconsequential for virulence. Abrogation of only extracellular siderophore biosynthesis caused only partial attenuation of virulence, indicating that RIA can partly compensate for the lack of siderophores in iron uptake during infection. Remarkably, deletion of *sid*D caused increased sensitivity to iron depletion and oxidative stress, and had a greate impact on virulence compared to *sid*F deletion. These differences suggested that abrogation of TAFC biosyntehsis at different steps of the pathway has different consequences; which might be related to accumulation of different pathway intermediates with differing consequences for the fungal metabolism. In agreement, the *sid*D mutant but not the *sid*F mutant was found to excrete the ultimate precursor of fusarinine C (Table-1), N^5-anhydromevalonyl-N^5-hydroxyornithine. *A. fumigatus* mutants producing fusarinine cinstead of TAFC due to deficiency in SidG (Fig. 1) displayed wild-type virulence, indicating that fusarinine C can satisfactorily replace TAFC as a siderophore not only in asenic growth but also during pathogenic growth. Individual abrogation of intracellular siderophore biosyntehsis by inactivation of SidC also caused partial attenuation of *A. fumigatus* virulence, which might be attributed to the role of FC in promoting germination and resisting oxidative stress. The partial rescue of virulence of the avirulent SidA-deficient mutant following reconstitution of the conidial hydroxyferricrocin content, which is possible by supplementation of the sporulation medium with FC, in the absence of de novo synthesis of both extracellular and intracellular siderophores demonstrates the importance of the conidial siderophore during the initial phase of infection.

Plant-pathogenic fungi
As noted, intracellular free iron is a potent source of highly cytotoxic reactive oxygen species, thus iron is tightly sequestered by iron-binding proteins, such as ferritin, in plant host cells, efficient iron uptake from host cells is a must for a successful phytopathogen. The role of microbial siderophores in virulence to plant hosts was first demonstrated for the bacterial pathogen *Erwinia chysanthemi*, which produces the catecholate chrysobactin and the carboxylate achromobactin. Comparison of wild-type and mutants deficient in chrysobactin-mediated iron uptake demonstrated that it is essential for systemic infection. Additionally, mutants defective in both chrysobactin and achromobactin biosynthesis are further attenuated in virulence. *Erwinia amylovora* synthesizes the hydroxamate desferrixamine, and mutants defective in desferrixamine biosynthesis show tissue specific reduced virulence, apple flower infection is reduced but seedling infection is not. Apart from these esamples of two *Erwinia* species, contributions of siderophores to bacterial infection of plant hosts have been demonstrated. For example, an exhaustive study of the possible role in virulence to tomato of the *Psudomonas syringae* pv. Tomato DC3000 mixed-type siderophore yersiniabactin and the fluorescent siderophore pyroverdin was reported recently. Although both siderophores were produced under iron limiting conditions, and the yersiniabactin was produced *in planta*, no differences in either bacterial growth or disease symptoms were observed between mutants ad wild-type strains inoculated on the plant host.

Symbiotic interactions
Siderophores are also important for maintaining mutualistic symbiotic associations of *Epichloe/Neotyphodium festucae* and grasses. Growth of the endophyte is confined to the intercellular spaces (Apoplast) of leaf sheaths and blades; the association is thought to be mutually beneficial for the plant and the fungus. *E. festucae* produces two siderophores, intracellular FC, and an extracellular fusarinine with a novel structure. NRPS2, an ortholog of the coprogen/fusarinine NRPSs, was found to be essential for extracellular siderophore biosynthesis and symbiosis, while inactivation of the FC NRPS had not effect on symbiosis.
Mycorrhizal symbisis positively affects the mineral nutrition of a wide range of terrestrial plants. Hydroxamate siderophore execration has been described for fungi involved in four types of mycorrhizal interaction: fusarinine C and FC by ericoid mycorrhizal species, FC by ectomycorrrhizal *Cenococcum geophilum*, FC by ectendomycorrhizal *Wilcoxina* spp. And *Phialocephala fortinii*, and basidiochrome (a novel trihydroxamate siderophore) by orchidaceous mycorrhizal *Ceratobasidium* spp. and *Rhizoctonia* spp. Siderophore production by these symbionts is believed, although not

progen, to contribute to the plant iron sypply. However, ectomycorrhizal *Laccaria bicolor* appears to lack hydroxamate siderophore biosynthesis.

Plant might benefit from fungal siderophores also by a different route because iron solubilized by hydrolysis products of fungal siderophores, e.g., fusarinines and dimerium acid, is a favourable iron source for plants.

The year *Debaryomyces mycophilus* spp. nov., isolated from the gut of woodlice species, differs from all other known fungal species by its inability to grow in culture without the presence of a siderophore (e.g., ferrichrome) of high amounts of iron normally not present in nature. This findings provides a new example for ecological interdependence.

Conclusion

- In recent years it became clear that the siderophore system constitutes a central element in iron homeostasis of many if not most fungi, affecting growth, oxidative stress resistance, as well as asexual and sexual development.
- Moreover, it became clear that siderophores are a common virulence determinant of at least some animal and plant pathogenic fungal species, and that siderophores are also involved in symbiosis.
- The fungal requirement for iron could potentially open up perspectives towards the development of novel antifungal treatments, e.g., iron chelation therapy or blocking of high-affinity iron acquisition or development of chemical surrogates for siderophores.
- Apart from applied exploitation of the knowledge on the fungal siderophore system, the challenge of the future will be the elucidation of several unexplored aspects, e.g., details of the siderophore biosynthetic pathway, the mechanism of siderophore excretion, the mode of iron release from intracellular siderophores and the interplay of iron metabolism, reactive oxygen species and fungal development, alone or in interactions with the host.

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