BioMetals **12:** 301–306, 1999.

Identification of a fungal triacetylfusarinine C siderophore transport gene (*TAF1*) in *Saccharomyces cerevisiae* as a member of the major facilitator superfamily

Petra Heymann[1], Joachim F. Ernst[2] & Günther Winkelmann[1,*]
[1]*Institut für Mikrobiologie & Biotechnologie, Universität Tübingen, Germany*
[2]*Institut für Mikrobiologie, Universität Düsseldorf, Germany*
[*]*Author for correspondence (Fax: +49 7071 2973094; E-mail: Winkelmann@uni-tuebingen.de)*

Received 15 September 1999; accepted 30 September 1999

Key words: iron, siderophores, transport, *Saccharomyces cerevisiae*, fungi

Abstract

Transport proteins of microorganisms may either belong to the ATP-binding cassette (ABC) superfamily or to the major facilitator (MFS)-superfamily. MFS transporters are single-polypeptide membrane transporters that transport small molecules via uniport, symport or antiport mechanisms in response to a chemiosmotic gradient. Although *Saccharomyces cerevisiae* is a non-siderophore producer, various bacterial and fungal siderophores can be utilized as an iron source. From yeast genome sequencing data six genes of the unknown major facilitator (UMF) family were known of which *YEL065w Sce* was recently identified as a transporter for the bacterial siderophore ferrioxamine B (Sit1p). The present investigation shows that another UMF gene, *YHL047c Sce*, encodes a transporter for the fungal siderophore triacetylfusarinine C. The gene *YHL047c Sce* (designated *TAF1*) was disrupted using the *kanMX* disruption module in a *fet3* background (strain DEY 1394 Δ*fet3*), possessing a defect in the high affinity ferrous iron transport. Growth promotion assays and transport experiments with ^{55}Fe-labelled triacetylfusarinine C showed a complete loss of iron utilization and uptake in the disrupted strain, indicating that *TAF1* is the gene for the fungal triacetylfusarinine transport in *Saccharomyces cerevisiae* and possibly in other siderophore producing fungi.

Introduction

Transport of iron in fungi proceeds mainly via siderophores which are biosynthesized under iron limitation (Van der Helm & Winkelmann 1994; Drechsel and Winkelmann 1997; Leong and Winkelmann 1998) In some fungi, like *Saccharomyces cerevisiae*, a reductive iron acquisition mechanism has evolved (Dancis *et al.* 1990, Askwith *et al.* 1994), which may be regarded as an additional iron acquisition system in order to extract iron from a variety of iron chelates with low stability constants. The property to transport siderophores has been retained in *S. cerevisiae* although biosynthesis of siderophores in this yeast does not occur (Neilands *et al.* 1987; Lesuisse & Labbe 1994). Triacetylfusarinine C (Figure 1), also named triacetylfusigen, is a common siderophore of many fungal genera and has been found in *Fusarium cubense* (Diekmann & Zähner 1967), *F. roseum* (Sayer & Emery 1968), *Aspergillus fumigatus* (Diekmann & Krezdorn 1975), *A. nidulans* (Charlang *et al.* 1988), *A. deflectus* (Anke 1977), *Mycelia sterilia* EP-76 (Adjimani & Emery 1987, 1988), *Penicillium resticulosum* (Konetschny-Rapp *et al.* 1988) and also in the basidiomycetous fungus *Agaricus bisporus*, the cultivated mushroom (Eng-Wilmot *et al.* 1992). Although non-acetylated forms as well as monomeric, dimeric and trimeric linear fusarinines have also been detected in fungal culture fluids (Winkelmann 1981; Drechsel & Winkelmann 1997), the cyclic acetylated trimer is regarded as the final biosynthetic siderophore product with the highest chemical stability. Earlier studies with

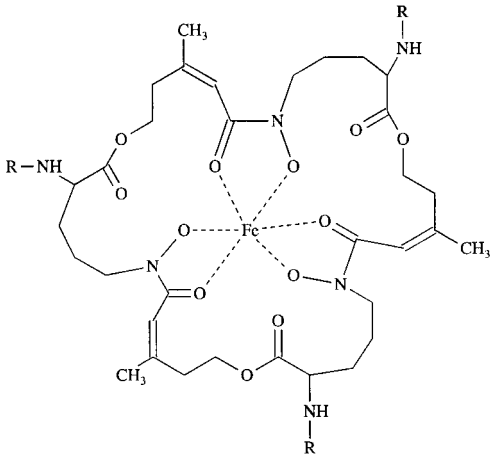

Fig. 1. Structural formula of N,N′,N′′-triacetylfusarinine C (triacetylfusigen) (R = CO-CH_3) and fusarinine C (fusigen) (R = H).

triacetylfusarinine C have shown that this siderophore is able to extract iron form other siderophores and thereby feeding the producing strain with iron from exogenous siderophores (Adjimani & Emery 1987). Most important is the observation that the three ester groups are easily hydrolyzed intracellularly leading to the excretion of various hydrolysis products into the culture fluid. Contrary to the above mentioned fungi, *S. cerevisiae* is unable to synthesize any hydroxamate siderophore as determined by the siderophore auxotrophic strain *Aureobacterium flavescens* JG9. Chrome azurole S (CAS) tests have also proven that *S. cerevisiae* does not synthesize any other siderophore (Neilands *et al.* 1987). We have shown earlier that overproduction of certain membrane proteins in *Neurospora crassa* under iron-deficient conditions does not occur (Huschka & Winkelmann 1989), indicating that there is no analogy to the iron regulated outer membrane proteins of bacteria (Braun & Hantke 1997). However, recent genome sequencing in *S. cerevisiae* showed that a variety of different transporters exist, which belong to the major facilitator superfamily; six putative MFS-proteins remained functionally uncharacterized and were therefore named unknown major facilitators (UMF) (Goffeau *et al.* 1997). While one of them (Yel065w Sce) could be recently assigned as a transporter for the bacterial ferrioxamine B (Lesuisse *et al.* 1998), we were able to show in the present investigation that a further UMF gene (*Yhl047c Sce*) encodes a transporter for the fungal triacetylfusarinine siderophore.

Materials and methods

Siderophores and HPLC

Triacetylfusarinine C (Triacetylfusigen), ferrichromes and ferrioxamines were from the stock of our laboratory. Fusigen was isolated from an ericoid mycorrhizal fungus (Haselwandter *et al.* 1992). Deferration of hydroxamate siderophores was according to Wiebe & Winkelmann (1997) using 8-hydroxyquinoline. The purity of siderophores was checked by HPLC using a reversed phase column (Nucleosil C18, 5 μm, 4 × 250 mm, Grom, Herrenberg, Germany) and an acetonitrile/water gradient (6–40%) with 0.1% trifluoroacetic acid (TFA) added to both solvents. HPLC separation was run on a HPLC (LC-10AT pumps, equipped with gradient controller and automatic sampler,Shimadzu, Duisburg, Germany). Detector wavelength was set at 435 nm or 220 nm which allowed to detect hydroxamate siderophores, desferrisiderophores and impurities.

Strains and culture conditions

Yeast strains were maintained on YPD (1% yeast extraxt, 2% peptone, 2% glucose) agar and grown overnight in liquid YPD medium at 30 °C under aeration on a rotary shaker.

Gene disruption

Disruption of the genomic MFS-encoding sequence (*YHL047c Sce* Accession No. AAB65059.1 GB) was accomplished by using the *kanMX* disruption module present on vector pUG6 (Wach *et al.* 1994; Güldener *et al.* 1996).

Amplification of the *kanMX* module by PCR was performed by using primers containing 3′ sequences homologous to the *kanMX* module (underlined) and 5′ sequences homologous to the *YHL047c Sce* gene (italics): YHL047c-L, 5′-*ATG ATC GAA GTC CCA GAA GAC AAT cgT TCT AGC CAA ACA A*ACA GCT GAA GCT TCG TAC GC-3′; YHL047c-R, 5′-*TCA GAG ATC ATT TTT TCG AAC ACA TAT CCT GTT GGG GTT T*GC ATA GGC CAC TAG TGG ATC TG-3′. Thus the primers contained genomic and disruption module sequences. The 1.5 kb PCR-fragment was checked by agarose gel electrophoresis.

Transformation

Competent cells of *S. cerevisiae* DEY1394 Δ*fet3* (Askwith *et al.* 1996) were transformed and positive clones were selected on YPD/G418 plates containing 200 μg/ml geneticin (G418, Calbiochem, Germany). The strain *S. cerevisiae* DEY1394 Δ*fet3*, kindly provided by David Eide, was used to exclude any high affinity reductive iron transport. The strain *S. cerevisiae* DEY1394 Δ*fet3* had the following characteristics: *MATα fet3::HIS3 ade6 can1 trp1 leu 2 his3 ura3*.

To verify correct integration of the disruption module into the *YHL047c Sce* gene control PCRs were performed with G418-resistant clones using the following primer pairs: YHL047c-A: 5′-CTG GAG TTT CTG TGC TCC-3′; YHL047c-D: 5′-GTA CTA CCG GAT TAG AGG-3′, and KanB: 5′-GGA TGT ATG GGC TAA ATG-3′ and KanC: 5′-CCT CGA CAT CAT CTG CCC-3′. Consistent with correct integration of the disruption module a 2054 bp fragment was obtained with primers YHL047c-A and YHL047c-D, a 481 bp fragment with primers YHL047c-A and KanB and 399 bp fragment with primers YHL047c-D and KanC.

Growth promotion assay

SD-softagar plates (0.67% yeast nitrogen base (Difco, Detroit), 2% glucose, 0.5% agar) were prepared containing yeast cells and 500 μM bathophenanthroline disulfonic acid (BPDS). Siderophores (10 μM) were pipetted on sterile filter disks (10 μl/disk), dried and placed on the agar plates. Growth zones were read after 24 and 48 h incubation at 30 °C.

Radioactive iron uptake

Strains were grown overnight in chemically defined medium (SD medium: 0.67% yeast nitrogen base (Difco, Detroit), 2% glucose, 0.5% agar). Transport kinetics were performed in SD medium containing 500 μM BPDS and 4 μM ^{55}Fe-triacetylfusarinine C. Triacetylfusarinine C was labeled with $^{55}FeCl_3$ (Amersham, UK, ^{55}Fe in 0.1 M HCl). For time dependent uptake studies cells (1 ml) were harvested at intervals by filtering through nitrocellulose filters and washed with 10 ml cold saline. Siderophore-bound iron taken up by the cells was measured by liquid scintillation counting.

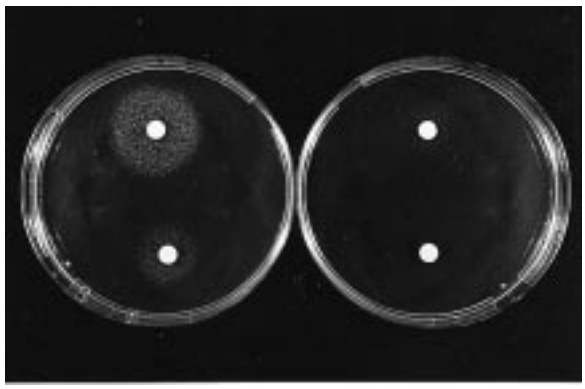

Fig. 2. Growth promotion test using *S. cerevisiae* DEY1394 Δ*fet3* (parental strain, left side) and DEY1394 Δ*fet3* Δtaf1 = PHY2 (disrupted strain, right side). Triacetylfusarinine C (10 μM) (upper) fusigen (100 μM) (*lower*).

Results

When the parental-strain (DEY1394 Δ*fet3*) and the disruptant (DEY1394 Δ*fet3* Δ*taf1* = PHY2) were compared in growth promotion tests containing various fungal and bacterial siderophores, growth was observed with all ferrichromes, coprogen and all bacterial ferrioxamines studied (Table 1). However, ferric triacetylfusarinine C (triacetylfusigen) and to a lesser degree fusigen, did not stimulate the growth of the disrupted strain as illustrated in Figure 2, indicating that the gene disruption had affected the function of iron utilization from fusarinine-type siderophores. Both compounds have a common cyclic ester structure but differ in the acetylated and free amino group of the fusarinine moieties. Thus, it can be assumed that the structure of anhydromevalonic acid linked to hydroxyornithine (fusarinine) is the recognized motif in the transported molecule.

In order to prove that the defect is locatlized in a transporter protein, additional transport experiments were performed using ^{55}Fe-labeled triacetylfusarinine C. As shown in Figure 3 short time transport kinetics (0–20 min) for uptake of radiolabeled ferric triacetylfusarinine C differed between both strains. Thus, triacetylfusarinine C-transport in the disrupted strain was significantly lower compared to the non-disrupted parental strain. Some residual binding of the labeled siderophore seems to occur, which may be caused by the fact that triacetylfusarinine is the most lipophilic siderophore among the fungal hydroxamate siderophores. The present data support the previous assumption that the *YHL047c Sce* gene encodes a siderophore transporter and have identified the trans-

Table 1. Growth promotion tests using fungal and bacterial hydroxamate siderophores and *S. cerevisiae* DEY1394 Δ*fet3* (parental strain) and DEY1394 Δ*fet3* Δtaf1 = PHY2 (disrupted strain)

Siderophores	DEY1394 Δ*fet3*	DEY1394 Δ*fet3* Δ*taf1*
Ferrichrysin	+++	+++
Ferricrocin	+++	+++
Ferrirubin	+++	+++
Ferrirhodin	+++	+++
Ferrichrome	+++	+++
Ferrichrome A	++	++
Ferrioxamine B	+++	+++
Ferrioxamine E	+++	+++
Coprogen	++	++
Triacetyfusarinine C	+++	−
Fusigen	+	−

Symbols: strong (+ + +), medium (++) and low (+) growth in the presence of added siderophores (10 μM).

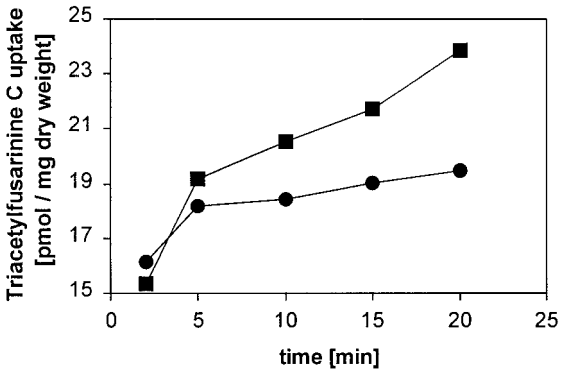

Fig. 3. Time-dependent transport of ^{55}Fe-triacetylfusarinine C (4 μM) by *S. cerevisiae* DEY1394 Δ*fet3*, parental strain (■) and DEY1394 Δ*fet3* Δtaf1 = PHY2, disrupted strain (●). Cells were grown overnight in SD-medium, sedimented and resuspended in SD medium, containing 500 μM BPDS. Aliquots of 1 ml cell suspension were taken at intervals, filtered, washed and the radioactivity was counted as described in Materials and methods. Uptake of ^{55}Fe-triacetylfusarinine C was calculated as pmoles per mg dry weight.

ported molecule. We therefore designate the *YHL047c Sc*e gene *TAF1*, for triacetylfusarinine C transporter. The *TAF1* gene product transports fusarinine-containing ester-type hydroxamate siderophores in *S. cerevisiae*. Similar transporters may exist in other siderophore-producing fungal genera.

Discussion

Triacetylfusarinine C is a typical fungal siderophore (Hossain *et al.* 1980). Its occurence in the *Aspergillus* and *Penicillium* group and the involvement of these fungi in pathogenesis (Howard 1999) underlines the importance of this siderophore. It is interesting to note that transport systems for fusarinines in bacteria are unknown so far (Braun & Hantke 1997). Although other fungal siderophores, such as ferrichromes and coprogen, are utilized by *Escherichia coli*, a fusarinine outer membrane receptor is still lacking.

A previous report has assigned the *SIT1* gene of the yeast *Saccharomyces cerevisiae* to the unknown major facilitator (UMF) gene *YEL065w Sce*, encoding a transporter for the bacterial siderophore ferrioxamine B (Lesuisse *et al.* 1998). Ferrioxamines are characteristic siderophores of Streptomycetes, but have also been detected in enterobacteria (Deiss *et al.* 1998).

In this investigation we present evidence that another gene of the UMF family, *YHL047c Sce*, is required for the transport of triacetylfusarinine C (triacetylfusigen), a typical fungal siderophore. As this UMF gene encodes a transporter for fusarinines (triacetylfusarinine C and fusigen) we have named the gene *TAF1*. Disruption of the *YHL047c Sce* gene was accomplished by a *kanMX* disruption module, containing homologous sequences derived from the *YHL047c* gene. To avoid iron uptake by the high affinity ionic iron uptake system (Dancis *et al.* 1990), a strain with a Δ*fet3* background was used for gene disruption. Selection on geneticin (G418) plates yielded mutants that were unable to utilize iron from ferric triacetylfusarinine C and fusigen in growth promotion tests. The defect in siderophore transport could be confirmed by performing transport measurements with

^{55}Fe-triacetylfusarinine C. Although it had been proposed earlier that the six UMFs identified in yeast (Pao *et al.* 1998) might be drug efflux pumps, the present investigation confirms their function in siderophore transport.

The mechanism of siderophore-iron transport in fungi has been dealt with in various studies and has been shown to be different depending on the type of siderophore transported (Van der Helm & Winkelmann 1994; Carrano *et al.* 1996,). In most cases the intact siderophore is taken up by the fungal cells and in some fungi enrichment of the ferric siderophore in the cytosol and transfer of desferri-siderophores in vacuoles has been shown (Ardon 1998).

We have previously shown that a membrane potential is required for siderophore transport in fungi (Huschka *et al.* 1983), driven by a proton-extruding ATPase. Moreover, hydroxamate siderophore transport seems to be directly coupled to an inward directed proton symport since no uptake occurs in alkaline medium (Huschka & Winkelmann 1984).

After having identified the transporter gene (*TAF1*) recognizing a fungal hydroxamate siderophore, further studies on the mechanism of siderophore transport in fungal membranes can now be resumed and offer new perspectives for an analytical approach. Thus, the previously found pronounced enantioselectivity of siderophore transport in fungal membranes (Winkelmann 1979, Winkelmann & Braun 1981; Münzinger *et al.* 1999) can now be studied by an interaction with the transporter protein. While most currently recognized MFS permeases are single polypeptides possessing a 12-transmembrane-spanner (TMS) protein topology, the UMFs possess 14 putative TMSs (Goffeau *et al.* 1997). Contrary to the ABC transporters which are capable of transporting also macromolecules, the MFS permeases are generally restricted to small molecules and some do transport small peptides. This also holds true for the fusarinine transporting Taf1 permease described here and the Sit1 permease (Lesuisse *et al.* 1998) which both transport low-molecular weight siderophores.

Another UMF gene (*YHL040c*) presently under study in our lab is essential for the transport of ferrichromes containing anhydromevalonic like residues (ferrirubin, ferrirhodin and ferrichrome A) (Heymann *et al.* 1999). The genes encoding the two UMF paralogs (Yhl047c and Yhl040c) cluster together and presumably result from an earlier gene duplication event (Pao *et al.* 1998). This assumption can now be functionally confirmed by showing that the anhydromevalonic motif in the fusarinines and in ferrirubin and ferrirhodin are essential elements in both gene products. Therefore a common recognition of the anhydromevalonic acid residue might be necessary for recognition and transport. We have shown earlier that in *Neurospora crassa* ferrirubin interferes with coprogen uptake, both of which contain anhydromevalonic acid residues (Huschka *et al.*1985, 1986). From multiple sequence alignments and phylogenetic trees of the proteins of the UMF family a high degree of sequence similarity was deduced suggesting that most if not all UMFs are involved in siderophore transport.

Acknowledgements

We thank David Eide and Andrew Dancis for providing strains, Michaela Gerads for technical assistance and Claudia Timpel for helpful advice in disruption and transformation techniques. This work was supported by a grant of the DFG.

References

Adjimani JP, Emery T. 1987 Iron uptake in *Mycelia sterilia* EP-76. *J Bacteriol* **169**, 3664–3668.

Anke H. 1977 Metabolic products of microorganisms 163. Desferritriacetylfusigen, an antibiotic from *Aspergillus deflectus. J Antbiotics* **30**, 125–128.

Askwith CC, De Silva D, Kaplan J. 1996 Molecular biology of iron acquisition in *Saccharomyces cerevisiae*. *Molec Microbiol* **20**, 27–34.

Braun V, Hantke K. 1997 Receptor-mediated bacterial iron transport. In: Winkelmann G, Carrano CJ, eds. *Transition Metals in Microbial Metabolism* Amsterdam, Harwood Academic Publisher.

Carrano C, Thieken A, Winkelmann G. 1996 Specificity and mechanism of rhizoferrin mediated metal ion uptake. *BioMetals* **9**, 185–189.

Dancis A, Klausner RD, Hinnebusch AG, Barriocanal JG. 1990 Genetic evidence that ferric reductase is required for iron uptake in *Saccharomyces cerevisiae*. *Mol Cell Biol* **10**, 2294–2301.

Deiss K, Hantke K, Winkelmann G. 1998 Molecular recognition of siderophores: A study with cloned ferrioxamine receptors (FoxA) from *Erwinia herbicola* and *Yersinia enterocolitica*. *BioMetals* **11**, 131–137.

Diekmann H, Zähner H. 1967 Konstitution von Fusigen und dessen Abbau zu Δ^2-Anhydromevalonsäurelacton. *Eur J Biochem* **3**, 213–218.

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: *Transition Metals in Microbial Metabolism*, Amsterdam, Harwood Academic Publishers, 1–49.

Eng-Wilmot DL, Adjimani JP, Van der Helm D. 1992 Siderophore-mediated iron(III) transport in the mycelia of the cultivated fungus *Agaricus bisporus*. *J Inorg Chem* **48**, 183–195.

Goffeau A, Park J, Paulsen IT, Jonniaux J-L, Dinh T, Mordant P, Saier MH. 1997 Multidrug-resistant transport proteins in yeast: complete inventory and phylogenetic characterization of yeast open reading frames within the major facilitator superfamily. *Yeast* **13**, 43–54.

Güldener U, Heck S, Fiedler T, Beinhauer J, Hegemann JH. 1996 A new efficient gene disruption cassette for repeated use in budding yeast. *Nucl Acid Res* **24**, 2519–2524.

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.

Heymann P, Ernst JF, Winkelmann G. 1999 Functional assignment of the unknown facilitator superfamily genes as membrane tranporter genes for fungal ferrichromes in *Saccharomyces cerevisiae* (submitted).

Howard DH. 1999 Acquisition, transport and storage of iron by pathogenic fungi. *Clin Microbiol Rev* **12,** 394–404.

Huschka H, Winkelmann G. 1984 A study on the mechanism of siderophore transport - a proton symport. *J Plant Nutr* **7**, 479–487.

Huschka, H. and Winkelmann, G. 1989 Iron limitation and its effect on membrane proteins and siderophore transport in *Neurospora crassa*. *BioMetals* **2**, 108–113.

Huschka H, Müller G, Winkelmann G. 1983 The membrane potential is the driving force for siderophore iron transport in fungi. *FEMS Microbiol Lett* **20**, 125–129.

Huschka H, Naegeli HU, Leuenberger-Ryf H, Keller-Schierlein W, Winkelmann G. 1985 Evidence for a common siderophore transport system but different siderophore receptors in *Neurospora crassa*. *J Bacteriol.***162**, 715–721.

Huschka H, Jalal MAF, Van der Helm D, Winkelmann G. 1986 Molecular recognition of siderophores in fungi: Role of iron-surrounding N-acyl residues and the peptide backbone during membrane transport in *Neurospora crassa*. *J Bacteriol* **167**, 1020-1024

Hossain MB, Eng-Wilmot DL, Loghry RA, Van der Helm D. 1980 Circular dichroism, crystal stucture, and absolute configuration of the siderophore ferric N, N′, N′′- triacetylfusarinine, $FeC_{39}H_{57}N_6O_{15}$. *J Am Chem Soc* **102**, 5766–5773.

Konetschny-Rapp S, Huschka H, Winkelmann G. 1988 High-performance liquid chromatography of siderophores from fungi. *BioMetals* **1**, 9–17.

Lesuisse E, Labbe P. 1994 Reductive iron assimilation in *Saccharomyces cerevisiae*. In: *Metal Ions in Fungi* Winkelmann G, Winge DR, eds. New York, Marcel Dekker Inc., 149–178.

Lesuisse E, Simon-Casteras M, Labbe P. 1998 Siderophore-mediated iron uptake in *Saccharomyces cerevisiae*: the SIT1 gene 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. In: Sigel H, Sigel A, eds. *Metal Ions in Biological Systems*, Vol. 35. New York, Marcel dekker, Inc.

Münzinger M, Taraz K, Budzikiewics H, Drechsel H, Heymann P, Winkelmann g, Meyer J-M. 1999 S,S-Rhizoferrin (enantiorhizoferrin) – a siderophore of *Ralstonia (Pseudomonas) pickettii* DSM 6297 – the optical antipode of R,R-Rhizoferrin isolated from fungi. *BioMetals* **12**, 189–193.

Neilands JB, Konopka K, Schwyn B, Coy M, Francis RT, Paw BH, Bagg A. 1987 Comparative Biochemistry of microbial iron assimilation. In: Winkelmann G, Van der Helm D, Neilands JB, eds. *Iron Transport in Microbes, Plants and Animals* VCH 1997, 3–33.

Pao SS, Paulsen IT, Saier MH Jr. 1998 Major facilitator superfamily. *Microbiol Molec Biol Rev* **62**, 1–34.

Sayer JM, Emery T. 1968 Structures of naturally occuring hydroxamic acids, fusarinine A and B. *Biochemistry* **7**, 184–190.

Van der Helm D, Winkelmann G. 1994 Hydroxamates and polycarboxylates as iron transport agents (siderophores) in fungi. In: Winkelmann G, Winge DR, eds. *Metal Ions in Fungi* New York, Marcel Dekker, Inc. 39–98.

Wach A, Brachat A, Pohlmann R, Philippsen P. 1994 New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae*. *Yeast* **10**, 1793–1808.

Wiebe C, Winkelmann G. 1975 Kinetic studies on the specificity of chelate-iron uptake in Aspergillus. *Arch Microbiol* **123**, 837–842.

Winkelmann G. 1979 Evidence for stereospecific uptake of iron chelates in fungi. *FEBS Lett.* **97**, 43–46.

Winkelmann G, Braun V. 1981 Stereoselective recognition of ferrichrome by fungi and bacteria. *FEMS Microbiol Lett* **11**, 237–241.

Winkelmann, G. (ed.) 1991 Handbook of Microbial Iron Chelates. Boca Raton, CRC Press Inc., FL.

Winkelmann G. 1993 Kinetics, energetics, and mechanisms of siderophore iron transport in fungi. In: Barton LL, Hemming BC, eds. *Iron Chelation in Plants and Soil Organisms* New York, Academic Press, 219–239.

Winkelmann G, Winge DR (eds.) 1994 *Metal Ions in Fungi*. New York, Marcel Dekker, Inc.