The Siderophore Iron Transporter of *Candida albicans* (Sit1p/Arn1p) Mediates Uptake of Ferrichrome-Type Siderophores and Is Required for Epithelial Invasion

Petra Heymann,[1,2] Michaela Gerads,[1] Martin Schaller,[3] Francoise Dromer,[4]
Günther Winkelmann,[2] and Joachim F. Ernst[1]*

Institut für Mikrobiologie, Heinrich-Heine-Universität Düsseldorf, Düsseldorf,[1] Institut für Mikrobiologie und Biotechnologie, Universität Tübingen, Tübingen,[2] and Dermatologische Klinik und Poliklinik für Dermatologie und Allergologie, Ludwig-Maximilian-Universität München, Munich,[3] Germany, and Unité de Mycologie Moléculaire, Institut Pasteur, Paris Cedex 15, France[4]

The human fungal pathogen *Candida albicans* contains a close homologue of yeast siderophore transporters, designated Sit1p/Arn1p. We have characterized the function of *SIT1* in *C. albicans* by constructing *sit1* deletion strains and testing their virulence and ability to utilize a range of siderophores and other iron complexes. *sit1* mutant strains are defective in the uptake of ferrichrome-type siderophores including ferricrocin, ferrichrysin, ferrirubin, coprogen, and triacetylfusarinine C. A mutation of *FTR1* did not impair the use of these siderophores but did affect the uptake of ferrioxamines E and B, as well as of ferric citrate, indicating that their utilization was independent of Sit1p. Hemin was a source of iron for both *sit1* and *ftr1* mutants, suggesting a pathway of hemin uptake distinct from that of siderophores and iron salts. Heterologous expression of *SIT1* in the yeast *Saccharomyces cerevisiae* confirmed the function of Sit1p as a transporter for ferrichrome-type siderophores. The *sit1* mutant was defective in infection of a reconstituted human epithelium as a model for human oral mucosa, while the *SIT1* strain was invasive. In contrast, both *sit1* and *SIT1* strains were equally virulent in the mouse model of systemic infection. These results suggest that siderophore uptake by Sit1p/Arn1p is required in a specific process of *C. albicans* infection, namely epithelial invasion and penetration, while in the blood or within organs other sources of iron, including heme, may be used.

The supply of iron limits the growth of pathogens within the human host. Body proteins including transferrin and lactoferrin sequester iron, and pathogens grow successfully only if they are able to acquire such bound iron. A frequent strategy used by microorganisms for iron acquisition is the secretion of siderophore iron chelators, which are able to withdraw iron from human protein-iron complexes because of their high affinities (18, 34). Siderophore-iron complexes are taken up by specific transport systems; however, microorganisms have also developed transport systems for heterologous siderophores produced in other species. For example, *Escherichia coli* possesses transporters not only for the homologous enterobactin (EB) and aerobactin siderophores but also for hydroxamate siderophores including ferrichrome (FCH), which are produced by fungi (34). Clearly, cross-species utilization of siderophores may be of ecological and medical importance, for example, in case of mixed infections in humans. The human host counteracts such microbial strategies, e.g., by lowering the iron content in circulating iron proteins such as transferrin (44).

Siderophore production and uptake has been demonstrated with many fungal species (18, 25, 45). However, the yeast *Saccharomyces cerevisiae* does not secrete siderophores, but it is able to take up ferric ions by a reduction system, which includes the Fre1p and Fre2p proteins (3, 6, 11); reduction at the cell surface is followed by the uptake of iron via the high- and low-affinity iron uptake systems. The multicopper oxidase Fet3p accepts ferrous ions generated by the Fre proteins, oxidizes them, and transfers the ferric ions to the membrane transporter encoded by *FTR1* (2, 42). The low-affinity uptake system consists of the *FET4* gene product (8). Alternatively, *S. cerevisiae* is able to take up siderophores produced by other microorganisms (27). Cell wall proteins Fit1p to Fit3p are required for the uptake of the siderophores FCH and ferrioxamine B (FoxB) but not triacetylfusarinine C (TAFC) and EB (32). Four transporters in the cytoplasmic membrane belonging to the major facilitator superfamily are relatively specific in their abilities to transport siderophores, e.g., Enb1p transports EB and Arn2p/Taf1p transports triacetylfusarinine and fusigen (15, 17, 48); other transporters are less specific, e.g., Sit1p transports ferrioxamine and FCH (26, 47) and Arn1p transports a wide range of hydroxamate-type siderophores (16, 48). The expression of genes encoding siderophore transporters is repressed by iron and by the high activity of the Tpk2p protein kinase A isoform (27, 35, 47, 48); the general transcriptional repressors Tup1p and Ssn6p contribute to the silencing of these loci (27). In contrast, in the absence of iron, the transcription factors Aft1p and Aft2p (4, 46) activate genes encoding siderophore transporters (47, 48).

Elements of a reductive iron uptake system similar to that of *S. cerevisiae* have been identified in the human fungal pathogen *Candida albicans.* The product of the *CaCFL95* gene, which is homologous to *S. cerevisiae* Fre proteins, was able to restore the growth of *fre* mutants, while an *FET3* homologue,

* Corresponding author. Mailing address: Institut für Mikrobiologie, Heinrich-Heine-Universität Düsseldorf, Universitätsstr. 1/26.12, D-40225 Düsseldorf, Germany. Phone and fax: 49 211 8115176. E-mail: joachim.ernst@uni-duesseldorf.de.

TABLE 1. Strains and plasmids used in this study

Strain or plasmid	Genotype or description	Reference or source
C. albicans strains		
CAF2-1	*URA3/ura3Δ::imm434*	10
CAI4	*ura3Δ::imm434/ura3Δ::imm434*	10
C4-SB1, C4-SB4	as CAI4, but *SIT1/sit1Δ::hisG-URA3-hisG*	This study
C4-SH1.1, C4-SH4.1	as CAI4, but *SIT1/sit1Δ::hisG*	This study
C4-SHB1.1	as CAI4, but *sit1Δ::hisG-URA3-hisG/sit1Δ::hisG*	This study
C4-SHH1.1	as CAI4, but *sit1Δ::hisG/sit1Δ::hisG*	This study
C4-SHB4.21	as CAI4, but *SIT1/sit1Δ::hisG-URA3-hisG/sit1Δ::hisG*	This study
C4-SHH4/21-VII	as CAI4, but *SIT1/sit1Δ::hisG/sit1Δ::hisG*	This study
C4-SHHB4/21-VII	as CAI4, but *sit1Δ::hisG-URA3-hisG/sit1Δ::hisG/sit1Δ::hisG*	This study
C4-SHHH4/21-VII	as CAI4, but *sit1Δ::hisG/sit1Δ::hisG/sit1Δ::hisG*	This study
SM1813C	integration of p1367-CaSITpORF/*Cla*I in C4-SHH1.1	This study
SM1814C	integration of p1367-CaSITp/*Cla*I in C4-SHH1.1	This study
Δ*Caftr1* (ura+)	as CAI4, but *ftr1Δ::hisG ftr1Δ::hisG ftr1Δ::hisG-URA3-hisG*	33
Fet-	as CAI4, but *fet3Δ::hisG fet3Δ::hisG-URA3-hisG*	9
S. cerevisiae strains		
DEY1394	*MATα ade6 can1 his3 leu2 trp1 ura3 fet3-2::HIS3*	2
PHY14	as DEY1394, but *arn1Δ::loxP arn2Δ::loxP sit1Δ::loxP enb1Δ::loxP-kanMX-loxP*	This study
C. albicans plasmids		
p1367/1	*URA3*-marked integrative vector	29
p1367-CaSITpORF	*SIT1* promoter and ORF in p1367	This study
p1367-CaSITp	*SIT1* promoter in p1367	This study
S. cerevisiae plasmids		
YCp22Gal	*GAL1/10* promoter in YCplac22	This study
YCp22Gal/SIT1	*SIT1* transcribed by *GAL1* promoter, in YCp22	This study

CaFET99, was not functional in the heterologous yeast (23). Two *C. albicans* homologues of *FTR1* were identified, of which *FTR1* was shown to be necessary for growth under iron-limiting conditions and for virulence (33). An *FET3* homologue was also identified, but it was not relevant for virulence (9). The standard strain used for gene disruptions in *C. albicans*, CAI4, has been reported to have a deletion of a gene next to the *URA3* locus, which is able to complement an *S. cerevisiae aft1* mutation but whose function in iron metabolism in *C. albicans* is unclear (13). Some reports have described the production of siderophores, especially of the hydroxamate type, by *C. albicans*; however, the nature of such siderophores has not been elucidated (20, 43). The uptake of siderophores, including FCH, has also been documented (1, 28, 30). Recently, a homologue of siderophore transporters in *C. albicans*, named CaArn1p/CaSit1p, has been characterized (1, 28). Its expression in *S. cerevisiae* was shown by Ardon et al. (1) to mediate uptake of FCH, but Lesuisse et al. (28) did not find transport activity and even demonstrated inhibition of FCH uptake in *S. cerevisiae* by CaArn1/CaSit1p. As in *S. cerevisiae*, the expression of genes encoding components of the reductive iron system and of *SIT1/ARN1* is induced in low-iron conditions and is repressed by Tup1p in *C. albicans* (28, 31).

The previously reported data on the Sit1p/Arn1p transporter left several questions unresolved, including (i) whether Sit1p/Arn1p was the only siderophore transporter in *C. albicans* and to what degree it contributed to the overall pattern of siderophore utilization and (ii) what the phenotype of a *SIT1/ARN1* deletion strain was with respect to *C. albicans* virulence. Both of these questions are addressed in the present study. We show that a group of siderophores of the FCH-type require

Sit1p/Arn1p for uptake, while others are independent of this transporter. The iron chelate hemin (HEM), which is found in the host in large amounts, is used as an iron source by *C. albicans* independent of Sit1p/Arn1p. Importantly, siderophore uptake mediated by Sitp/Arn1p is required in a specific process during the infection of humans, namely the infection of epithelial layers, while infections of the bloodstream cause disease independent of Sit1p/Arn1p.

MATERIALS AND METHODS

Strains and growth conditions. Yeast strains (Table 1) were grown in yeast extract-peptone-dextrose (YPD) medium or on synthetic dextrose (SD) or synthetic galactose (SGal) minimal medium at 30°C (41). SD medium contained 2% glucose and SGal medium contained 2% galactose and 0.1% glucose as carbon sources. The solid and liquid media used to test hyphae formation of strains were as described previously (5). Transformation of *C. albicans* was carried out by the spheroplast method and that of *S. cerevisiae* was carried out by the salt method (41).

To construct an *S. cerevisiae* strain lacking all known siderophore transporters, successive deletions in the *fet3* mutant DEY1394 (2) were made with the *loxP-kanMX-loxP* module by using PCR fragments as described previously (14). *ARN1* (16, 48), *ARN2/TAF1* (15, 48), *SIT1* (26, 47), and *ENB1* (17) were deleted, with the *kanMX-loxP* sequence removed in the first three cases by the Cre recombinase, to allow subsequent disruptions. The correct insertion of the disruption fragments was verified by diagnostic PCRs on genomic DNA, with primers external and internal to the disruption fragments (data not shown).

Siderophores and siderophore biotests. Siderophores were isolated from microbial cultures as described previously, and the purity of the siderophores was checked by high-pressure liquid chromatography (15, 16, 17). In addition, FCH was purchased from Sigma.

The solid media used in the *C. albicans* siderophore growth tests were Lee's medium containing 1% agar (24) and 550 μM bathophenanthroline disulfonic acid (BPDA) as an iron chelator. Twenty to forty microliters of an overnight culture, in Lee's medium, of the yeast strain to be tested was added to 10 ml of molten medium, mixed, and allowed to solidify in petri dishes. Ten microliters of

siderophore solution (100 µM) or of other iron chelates (1 mM ferric citrate [CIT], 300 µM HEM) was dropped on sterile filter disks, which were placed on the agar surface. Plates were incubated for 2 to 4 days at 37°C. Siderophore growth tests for *S. cerevisiae* were performed similarly, except that SGal medium was used for pregrowth of cells and SGal agar containing 200 µM BPDA was used and siderophores were at a concentration of 10 µM, with the exception of EB and coprogen (COP) (100 µM).

Disruption of *SIT1*. *SIT1* alleles were deleted by the *URA* blaster protocol (10). To generate a disruption fragment for *SIT1*, the 5′ and 3′ untranslated regions of *SIT1* were first produced by genomic PCR using DNA of strain CAI4. The 5′ region (1.533 kb) was amplified by using primers SITdis1/for (5′-ATT*A-GATCT*TTGATACGCAGGAG-3′) and SITdis1/rev (5′-ATT*GGATCC*TAA-GATGTCATGCTAGC-3′), and the 3′ region (595 bp) was amplified by using primers SITdis2/for (5′-ATT*AGATCT*CAAACTTGGTTGTAAC-3′) and SIT-dis2/rev (5′-ATT*GGATCC*TGTAAGTAGTGATTACTG-3′) (newly introduced *Bgl*II and *Bam*HI sites are underlined; regions of homology are in italic type). Both fragments were introduced into the *Bgl*II or *Bam*HI site flanking the *URA* blaster module (*hisG-URA3-hisG*) of plasmid p5921 (10) to generate plasmid pSITdisBlaster. From this plasmid, a 6.14-kb *Bgl*II-*Bam*HI disruption fragment was excised, which was used for transformations of *ura3 C. albicans* strains. A 1.161-kb *Kpn*I fragment of a pUC18 subclone of the 5′ untranslated fragment (plasmid pSIT-disI) was used as a probe in Southern blottings.

Reconstitution of *SIT1* in the *sit1* mutant. Reconstituted strains were constructed in which the *sit1* mutation in one chromosomal allele of strain C4-SHH1.1 was reconstituted (SM1813C) or not reconstituted (SM1814C) and which contained the *URA3* allele in the same position, within the *SIT1* locus. An *Spe*I fragment containing the *SIT1* promoter and open reading frame (ORF) regions was generated first by genomic PCR using primers contig prom+*Spe*I (5′-TTA*ACTAGT*CATCTACGAGACTG-3′) and contig stop+*Spe*I (5′-TTA-*ACTAGT*TCTAAACAGCTACTC-3′) (introduced *Spe*I sites are underlined; regions of homology are in italic type); the *Spe*I site at the 5′ end of the promoter segment is at position −1057 relative to the ATG start sequence of the ORF. This fragment was introduced into pUC18 to generate pPromSit1 (1). The 2.873-kb *Spe*I fragment of this plasmid was inserted into the single *Xba*I site of plasmid p1367/1 (29) to generate the *URA3*-containing integration vector p1367-CaSITpORF. pPromSit1 (1) was also doubly digested with *Spe*I/*Nhe*I, and the 1.051-kb *Spe*I/*Nhe*I *ARN1* promoter fragment was isolated (the *Nhe*I site is 6 bp upstream of the ATG translation initiation sequence of *SIT1*) and inserted into the *Xba*I site of p1367/1 to generate the control plasmid p1367-CaSITp. Both p1367/1 derivatives were cut within the *SIT1* promoter at the single *Cla*I site (situated at position −851 relative to the ATG) and transformed into the *sit1* mutant C4-SHH1.1. The correct integration of plasmid p1367-CaSITpORF at the *SIT1* locus was verified by Southern blottings by using genomic DNA digested with *Ehe*I, which cuts genomic DNA at position −1766 relative to the ATG, within pUC18 sequences of the p1367/1 derivatives and within the *hisG* sequence. As a probe, the 640-bp *Hin*dIII-*Bgl*II fragment of plasmid pSIT-disI was used. In cases of correct integration of p1367-CaSITpORF in one *arn1* allele (about 60% of transformants), the reconstituted *SIT1* gene was apparent as two fragments of 5.123 and 4.419 kb (the latter fragment appeared weak on blots because of low homology to the probe), while the *hisG*-disrupted allele yielded a 2.63-kb fragment. The correct integration of the control plasmid p1367-CaSITp (lacking the *SIT1* ORF) was verified by Southern blottings by using genomic DNA digested with *Ssp*I and by using a 515-bp *Ssp*I/*Hin*dIII probe derived from plasmid pSIT-disI. The starting mutant p1367-CaSITp generated a single fragment of 3.5 kb, while in the case of an integrated plasmid, two fragments of 1.814 and 5.2 kb were observed because of an *Ssp*I site located within pUC18 sequences; in CAI4, the expected genomic fragment of 3.9 kb was verified.

Construction of an *S. cerevisiae* expression vector for *CaSIT1*. The Ca*SIT1* ORF was amplified by PCR on genomic DNA of strain CAI4 by using primers Contig4-3057/for (5′-TTA**GGATCC**TAGC<u>ATG</u>ACATCTTACCAGTC-3′) and Contig4-3057/rev (5′-TTA**GGATCC**TAGTT<u>CTA</u>AACAGCTACTC-3′) (newly introduced *Bam*HI sites are in boldface type, start and stop sequences are underlined, and regions of homology are in italic type). The 1.842-kb *Bam*HI fragment was inserted into the transformation vector YCplac22Gal (*Bam*HI cut), placing *CaSIT1* in the resulting plasmid YCp22Gal/SIT1 under the control of the *GAL1* promoter. YCplac22Gal was constructed by inserting the *Bam*HI-*Eco*RI fragment carrying *GAL1* (22) into YCplac22 (12).

In vitro model of oral candidiasis. The human epithelium for the in vitro model of oral candidiasis was supplied by Skinethic Laboratory (Nice, France). Human keratinocytes derived from a squamous cell carcinoma of the buccal mucosa (cell line TR146) were cultured on an inert supporting membrane (37). A defined medium based on the MCDB-153 medium (Clonetics, San Diego, Calif.) supplemented with 5 µg of insulin ml^{-1} was deposited below a 0.5-cm^2 microporous polycarbonate filter (pore size, 0.5 µm) and incubated for 7 days at 37°C with 100% humidity and 5% CO_2 to generate a human reconstituted epithelium (RHE). No antibiotics and antimycotics were used.

C. albicans strains were cultured for 24 h at 37°C on Sabouraud dextrose agar (Difco). A part of the culture was suspended in a 0.9% sodium chloride solution and centrifuged. After the suspension was washed three times, 2×10^5 cells were suspended in 10 ml of YPD medium. The suspension was cultured for 16 h at 25°C, 4×10^6 cells were suspended in fresh medium, and the suspension was incubated again for 24 h at 37°C with shaking. After the suspension was washed three times with phosphate-buffered saline (PBS) solution, the desired inoculum size was adjusted with PBS.

RHE (0.5 cm^2) was infected with 2×10^6 cells of each *C. albicans* strain in 50 µl of PBS; as a control, 50 µl of PBS alone was used. RHE was incubated at 37°C with 5% CO_2 at 100% humidity for 6, 12, and 22 h. Infected RHE samples were examined by light microscopy as described previously (39).

Mouse infections. Six-week-old BALB/c male mice were housed 10 per cage and given food and water ad libitum. The *C. albicans* strains to be tested were subcultured on SD agar medium for 18 h at 30°C before inoculation; strain identities were encoded to secure unbiased experimentations. The yeast colonies were scraped off the plates and washed three times in sterile saline. The inoculum was adjusted to a concentration of 10^7 cells/ml by microscopy countings, and 100 µl of the suspension was injected into the lateral tail vein of each mouse. The viability of the yeast cells was assessed by plating appropriate dilutions of the inoculum onto Sabouraud dextrose agar. Survival was monitored once daily up to day 68 after inoculation. Relevant results were confirmed thrice.

RESULTS

Disruption of *SIT1/ARN1*. The sequence of the *C. albicans* genome (obtained from the Stanford Genome Technology Center website [http://sequence-www.stanford.edu/group/candida]) revealed an ORF (Contig6-2443 complementary strand, coordinates 14150 to 15964) encoding a theoretical protein of 604 amino acids with high homology to *S. cerevisiae* siderophore transporters. Computer predictions suggested a membrane protein of nine transmembrane regions which is 51% identical to Arn1p, 46% identical to Sit1p, 44% identical to Arn2p/Taf1p, and 32% identical to Enb1p of *S. cerevisiae*. While this present study was in progress, Ardon et al. (1) and Lesuisse et al. (28) reported that this gene, designated *CaARN1* or *CaSIT1*, respectively, is regulated by iron in *C. albicans* and that its expression permits or inhibits FCH uptake in *S. cerevisiae*. Here, this gene is referred to as *SIT1* (alias *ARN1*), because (i) the designation *SIT* (siderophore iron transport) is descriptive of its function, (ii) a close homologue of *AFT1* is lacking in *C. albicans* genomic sequences and thus makes the designation *ARN* (Atf1 regulated) meaningless, and (iii) it is the only close homologue in *C. albicans* of the four known siderophore transporters of *S. cerevisiae*.

We successively disrupted *SIT1* alleles of *C. albicans* by the *URA* blaster method (10). The 5′ and 3′ untranslated regions of *ARN1* were produced by genomic PCRs and inserted next to the *hisG-URA3-hisG* blaster module to construct a disruption fragment, which was able to integrate into the *SIT1* locus by homologous recombination (Fig. 1A, top). *ura3* derivatives of disruptants were isolated and used for subsequent rounds of gene disruptions with the disruption fragment, as described previously (10). Correct insertion and disruption of *SIT1* was verified by Southern blottings able to distinguish the wild-type allele (*SIT1*), the *URA* blaster-disrupted allele (*sit1*Δ:: *hisG-URA3-hisG*), and the *URA3*-free allele (*sit1*Δ::*hisG*) (Fig. 1A, bottom) (see Materials and Methods). This procedure led to the disrupted strain C4-SHB1.1, which was disrupted in two *SIT1* alleles (Table 1) (Fig. 1A, bottom, lane 5). Surprisingly, in
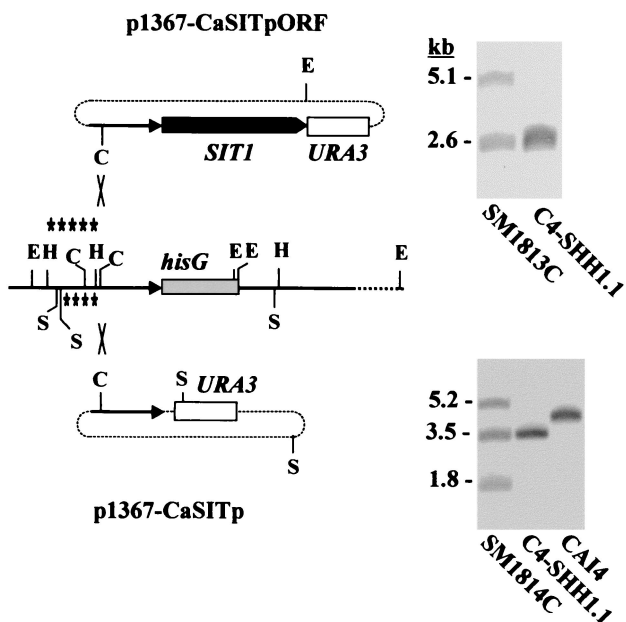
A.
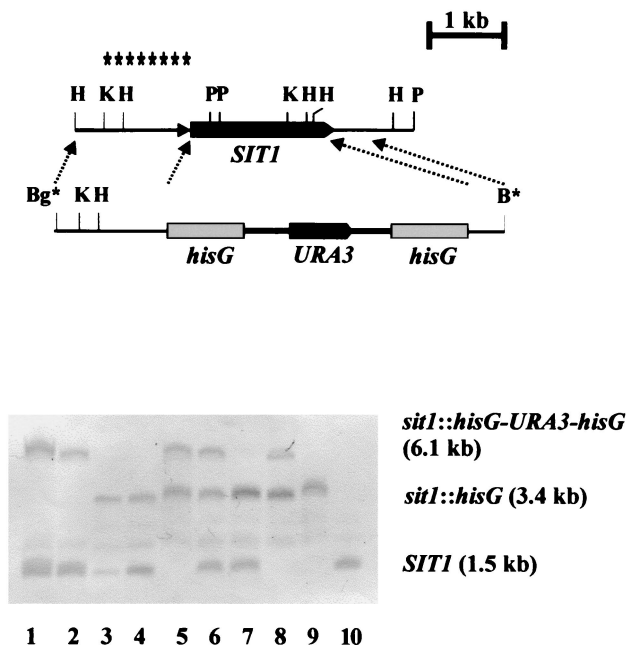
B.

FIG. 1. Disruption of *SIT1*. (A) The wild-type genomic structure of *SIT1* and the disruption fragment containing the *URA* blaster module (*hisG-URA3-hisG*) are shown (top). The relevant diagnostic restriction sites at the examination of the genomic configuration of *SIT1* are *Hin*dIII (H), *Kpn*I (K), and *Pvu*II (P); the *Bgl*II (Bg*) and *Bam*HI (B*) sites at the ends of the disruption fragment were generated by PCR. Asterisks indicate the region used as a probe in the Southern blot (bottom). Genomic DNAs of the following strains were digested by *Kpn*I and *Pvu*II and analyzed by Southern blotting: C4-SB1 (lane 1)

another lineage, the presence of a third *SIT1* allele was detected, becoming apparent after disruption of the presumed second *SIT1* allele in strain C4-SHB4.21; in this case, all three possible alleles, *SIT1/sit1Δ::hisG-URA3-hisG/sit1Δ::hisG*, occurred (Fig. 1A, bottom, lane 6). Because a regular diploid disruptant was obtained, we consider the occurrence of three *SIT1* alleles in strain C4-SHB4.21 as an artifact generated during the disruption processes.

To ensure that any potential phenotype observed in the *sit1* mutant was due to the lack of *SIT1* and not to other details of genetic configurations (e.g., any locus-dependent differences in *URA3* expression between the mutant and wild-type strains, which can occur [L. L. Sharkley, W.-L. Liao, and W. A. Fonzi, Abstr. EuroConference on Fungal Virulence Factors and Disease, abstr. p. 21, 2001]), we constructed two closely related strains either containing or lacking *SIT1* (strains SM1813C and SM1814C, respectively). To accomplish this, plasmid p1367-CaSITpORF (containing the *SIT1* promoter and coding region) or plasmid p1367-CaSITp (containing only the *SIT1* promoter) was cut at the single *Cla*I site within the promoter to stimulate integration into the genomic *SIT1* promoter in strain C4-SHH1.1 (*sit1Δ::hisG/sit1Δ::hisG*) (Fig. 1B). The correct integration of both plasmids in single copy at the *SIT1* locus was verified by Southern blottings (Fig. 1B). Thus, the resulting strains SM1813C and SM1814C both contain *URA3* at the *SIT1* locus and differ only in the presence of the *SIT1* coding region.

No growth defect was observed in the homozygous *sit1* mutant C4-SHB1.1 compared with the wild-type or reconstituted *SIT1* strain. Similarly, the formation of hyphae on solid media (Lee's medium, Spider medium, and serum-containing medium) or in liquid inducing media (in the presence of GlcNAc or serum) was not affected. The only exception was the triple allelic *sit1* mutant C4-SHHB4/21-VII, which in YPD and SD media had a growth rate reduced by about 20% compared to those of *SIT1* strains and mutant C4-SHB1.1 and which failed to form hyphae. Thus, these results suggest that an increased gene dosage or other genetic rearrangements interfere with growth and hyphal morphogenes in the triple allelic mutant but that these phenotypes are not related to the lack of *SIT1*.

Siderophore uptake by *C. albicans sit1* mutants. To establish the specificity of the theoretical Sit1p transporter protein, we compared the ability of *SIT1*^{+} and *sit1* mutant strains to take up various siderophores or other iron-containing compounds.
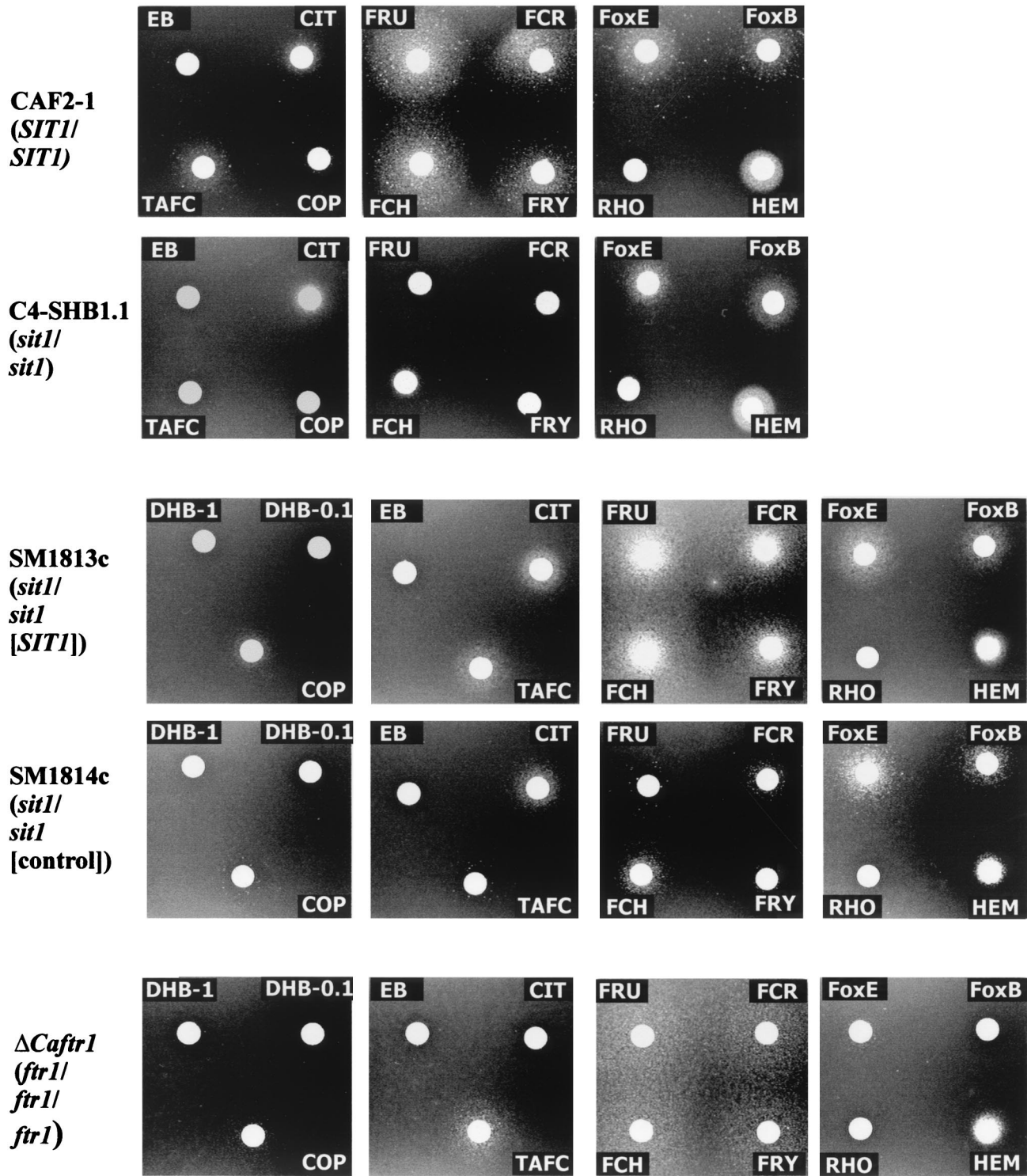
FIG. 2. Utilization of siderophores by *C. albicans* strains. The indicated strains were embedded in solid Lee's medium containing 550 μM BPDA, and sterile filter paper disks containing siderophores (10 μl) were placed on the agar surface. Plates were incubated at 37°C for 2 to 3 days and photographed. The concentrations of siderophores and other compounds are listed in Table 2.

Strains were embedded in Lee's medium containing 550 μM BPDA to make remaining traces of iron unavailable to *C. albicans* cells; growth on these plates was stimulated by the presence of siderophores or other iron compounds within the filter disks (Fig. 2).

In the first set of experiments, we compared the wild-type strain CAF2-1 with the *sit1* mutant C4-SHB1.1. The hydrox-amate siderophores FCH, ferricrocin (FCR), ferrichrysin (FRY), and ferrirubin (FRU) were excellent iron sources for the *SIT1*^{+} strain, but these siderophores were hardly used by

TABLE 2. Siderophore uptake by *C. albicans* strains

Siderophore or other compound[a]	Growth of C. albicans strains[b]					
	CAF2-1 (*SIT1/SIT1*)	C4-SHB1.1 (*sit1/sit1*)	SM1813c (*sit1/sit1* [*SIT1*])	SM1814c (*sit1/sit1* [control])	Δ*Caftr1* (*ftr1/ftr1/ftr1*)	Fet- (*fet3/fet3*)
COP	+	−/+	+	−/+	−/+	+
EB	−	−	−	−	−	−
FCH	+++	−/+	+++	−/+	+++	+++
FCR	+++	−/+	+++	−/+	+++	+++
FRY	+++	−/+	+++	−/+	+++	+++
FRU	+++	−/+	+++	−/+	+++	+++
FoxB	+	+	+	+	−	+
FoxE	++	+	++	++	−/+	++
RHO	−	−	−	−	−	−
TAFC	+	−/+	+	−/+	+	+
CIT	+	+	+	−/+	−	+
DHB	ND	ND	−	−	−	−
HEM	+	+	+	+	+	+

[a] The concentrations were 100 μM for the siderophores, 1 mM for DHB, 1 mM for CIT, and 300 μM for HEM. Siderophore solutions (10 μl) were added to sterile filter disks and placed on solid Lee's medium containing 550 μM BPDA.
[b] Growth zone diameters in agar diffusion tests: +++, >30 mm; ++, 20 to 30 mm; +, 10 to 20 mm; −/+, up to 10 mm. ND, not determined.

the *sit1* mutant. Compared to these siderophores, the hydroxamates TAFC and COP were less efficiently used by the wild-type strain, but in the presence of an *sit1* mutant background, siderophore utilization declined further. In contrast, ferrioxamine E (FoxE) and to a lesser extent FoxB were used as iron sources, but the uptake of these hydroxamates was not affected by the *sit1* mutation. Similarly, the uptakes of CIT and of HEM were not affected by the *SIT1* status of cells. Other (iron-free) siderophores used in these assays, including EB, dihydroxybenzoic acid (DHB), or rhodotorulic acid (RHO), did not stimulate growth. An identical iron chelate utilization pattern was obtained for strain C4-SHHB4/21-VII, which contains three *sit1* alleles (data not shown). Thus, these results suggested that the Sit1p transporter is essential for the uptake of a group of hydroxamate siderophores, especially of the FCH type, while the uptake of ferrioxamines, CIT, and HEM occurs irrespective of the presence of Sit1p.

This conclusion was confirmed by analysis of a carefully matched pair of strains, SM1813c and SM1814c, which differ only in the presence of the *SIT1* coding region, while all other genetic markers including the *URA3* gene are in identical locations. The above-mentioned patterns of siderophore utilization were again obtained with respect to (i) FCH, FCR, FRY, and FRU; (ii) TAFC and COP; (iii) FoxE and FoxB, CIT and HEM; and (iv) EB, DHB, and RHO (Fig. 2).

C. albicans ftr1 and *fet3* mutants defective in the reductive iron uptake system have been described previously (9, 33) but have not yet been tested for uptake of the range of siderophores as described here. Siderophore and iron chelate uptake in the *fet3* mutant was indistinguishable from that in any *SIT1* or *sit1* mutant strain (data not shown). In contrast, in the *ftr1* mutant, the uptake of the apparent Sit1p substrates FCH, FCR, FRY, FRU, TAFC, and COP still occurred as in the wild type, but the uptake of FoxE, FoxB, and CIT was prevented. Thus, the results summarized in Table 2 indicate that the uptake of the latter substrates depends on Ftr1p and is independent of the Sit1 protein, which we can consider as the main hydroxamate siderophore transporter of *C. albicans*.

Heterologous expression of *CaSIT1* in *S. cerevisiae*. While this present study was in progress, two reports on the expression of *CaARN1/SIT1* in *S. cerevisiae* appeared, one of which described the functional expression of *ARN1/SIT1* (1), which is at variance with the results of the second report (28). In our study, we prevented the significant activity of resident iron transporters by constructing an *S. cerevisiae* host strain (PHY14) defective in elements of reductive iron uptake (*fet3*) and lacking all four known transporters of siderophores, Arn1p, Arn2p/Taf1p, Sit1p, and Enb1p. This strain was unable to grow on low-iron media, even in the presence of most siderophores, while the parental strain DEY1394 (2) appeared to utilize siderophores as an iron source. PHY14 was transformed with an expression vector for *CaSIT1* (pYCp22Gal/SIT1) in which the coding region of this gene was transcribed by the *GAL1* promoter. Transformants were tested for their utilization of siderophores in the plate assay by using galactose as the carbon source to induce expression of the *GAL1-SIT1* fusion. The results are shown in part in Fig. 3 and summarized in Table 3.

Hydroxamate siderophores of the FCH type, including FCH, FCR, FRY, and FRU, as well as COP and TAFC, allowed the growth of *S. cerevisiae* PHY14(pYCp22Gal/SIT1) on galactose-containing media but not on glucose media, which repressed the *GAL1-SIT1* fusion gene. In addition, the growth of ferrioxamine-type siderophores such as FoxE or FoxB was not improved by expression of *CaSIT1*. These results reflected the above-mentioned siderophore specificities obtained for CaSit1p in *C. albicans* and strongly suggested that these specificities were due to CaSit1p itself and not to another component, such as a resident transporter stimulated by CaSit1p.

In vitro model of oral candidiasis. The keratinocyte cell line TR146 was allowed to differentiate on an inert supporting membrane, and as a result, a multilayered keratinocyte tissue was generated, which is strikingly similar to human oral mucosa (Fig. 4). This RHE was infected with 2×10^6 cells of *C. albicans* strains (yeast form) to explore any function of Sit1p in the infection process.
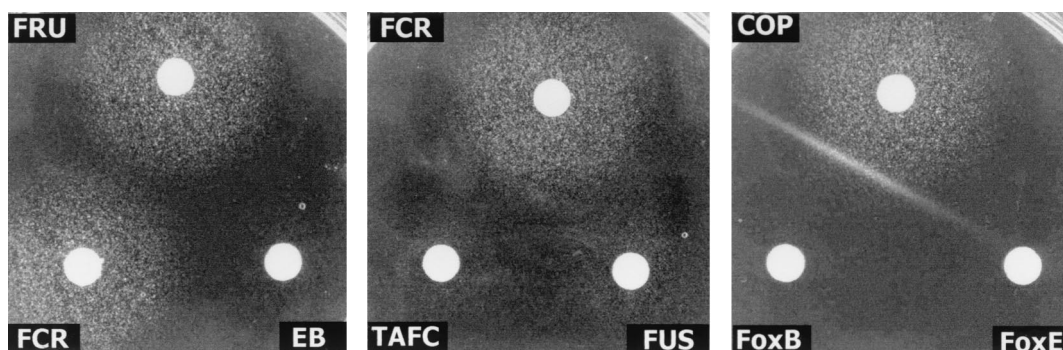
FIG. 3. Utilization of siderophores by *CaSIT1* transformant of *S. cerevisiae*. Strain PH14(YCp22Gal/SIT1) was embedded in solid SGal medium containing 200 μM BPDA, and sterile filter paper disks containing siderophores (10 μl) were placed on the agar surface. Plates were incubated at 30°C for 2 to 3 days and photographed. See Table 2 for concentrations. FUS, fusigen.

Inspection of the RHE by light microscopy revealed that infection by the *sit1* mutant C4-SHB1.1 at an early time point (6 h) did not result in any noticeable damage to the RHE, and even at later time points, few *C. albicans* cells were observed in the upper layer of keratinocytes. In contrast, the *SIT1*^{+} strain CAF2-1 had entered the upper cellular layers at 6 h and both hyphae and yeast form cells were visible. At 22 h after infection, the *SIT1*^{+} strain had caused extensive damage to the RHE, which was apparent as large cavities in the keratinocyte tissue. These results were confirmed by use of the matched pair of transformants, strains SM1813c (SIT1) and SM1814c (*sit1*). While SM1814c had hardly damaged the RHE at 12 h after infection, hyphal forms of SM1813c had penetrated to the deepest keratinocyte layers at this time point (Fig. 4).

Thus, these results suggest that in a specific phase of infection of the human host by *C. albicans*, i.e., the invasion and penetration of the mucosal epithelium, siderophore uptake by Sit1p is required.

Virulence tests with mice. The virulence of *sit1* mutants was compared with that of *SIT1*^{+} strains in the standard mouse model of systemic infection. BALB/c mice were injected in their tail veins with 10^6 yeast form cells, and the number of surviving mice was recorded. In a representative experiment, the *sit1* mutant C4-SHB1.1 was able to kill mice as fast as the wild-type strain CAF2-1 or the *SIT1*/*sit1* half-disruptant strain

C4-SB1 (Fig. 5). In contrast, the abnormal strain C4-SHH4/21-VII, containing three disrupted *SIT1* alleles, was completely avirulent, and mice survived for 68 days following their inoculation. However, because the *sit1* mutant is virulent, it appears that the Sit1 siderophore transporter is not essential for survival and virulence if *C. albicans* cells are injected into the bloodstream. HEM and other iron-containing compounds such as transferrin may be used as sources of iron in the blood and organs (Fig. 2). The avirulence of the triple-allele mutant must be explained by genetic alterations unrelated to the absence of *SIT1*, such as the effects on virulence caused by increased gene dosages.

DISCUSSION

The construction of *C. albicans sit1* mutants and their comparison to *SIT1*^{+} strains allowed us to verify the role of the encoded protein Sit1p in siderophore utilization and virulence. With respect to siderophore utilization, we found by using a range of iron chelates including siderophores that Sit1p is needed for the uptake of hydroxamate-type siderophores, not only for that of FCH as previously suggested (1) but also for that of related siderophores including FCR, FRY, FRU, COP, and TAFC. The latter two siderophores were taken up less efficiently than the former siderophores, but their utilization was clearly dependent on Sit1p. Unexpectedly, *sit1* mutants were still able to the use FoxE and FoxB, suggesting the presence of a siderophore transporter other than Sit1p in *C. albicans*. Analysis of the recently reported genomic sequences of *C. albicans* (contig 6) revealed two distantly related homologues of Sit1p encoding putative facilitators; the theoretical transporter proteins encoded by ORF6.8902 and ORF6.1908 have 25 and 22% identity, respectively, to Sit1p. Interestingly, the Sit1p-independent uptake of ferrioxamine siderophores was dependent on the function of the reductive iron transport system, since an *ftr1* mutant (33) was unable to utilize ferrioxamines as sources of iron. In *S. cerevisiae*, reductive iron uptake requires both Ftr1 and Fet3 proteins (2, 42). Since a *C. albicans fet3* mutant lacking the second possible element of reductive iron import (9) was not blocked in ferrioxamine import, it appears that homologues of Fet3p, which are indeed predicted from the *C. albicans* genomic sequences, have functions that are redundant to those of Fet3p. The *ftr1* mutant was also

TABLE 3. Siderophore uptake by *S. cerevisiae* strains

Siderophore[a]	Growth of *S. cerevisiae* strains[b]		
	DEY1394	PHY14	PHY14 (YCp22Gal/SIT1)
FoxB	++	−	−
FoxE	++	−	−
EB	++	−	−
TAFC	++	−	+/−
FRY	++	−	++
FCR	++	−	++
FCH	++	−	++
FRU	++	−	++
Ferrirhodin	++	−	++
COP	+	−	++

[a] Siderophore concentrations were 10 μM, except for EB and COP (100 μM).
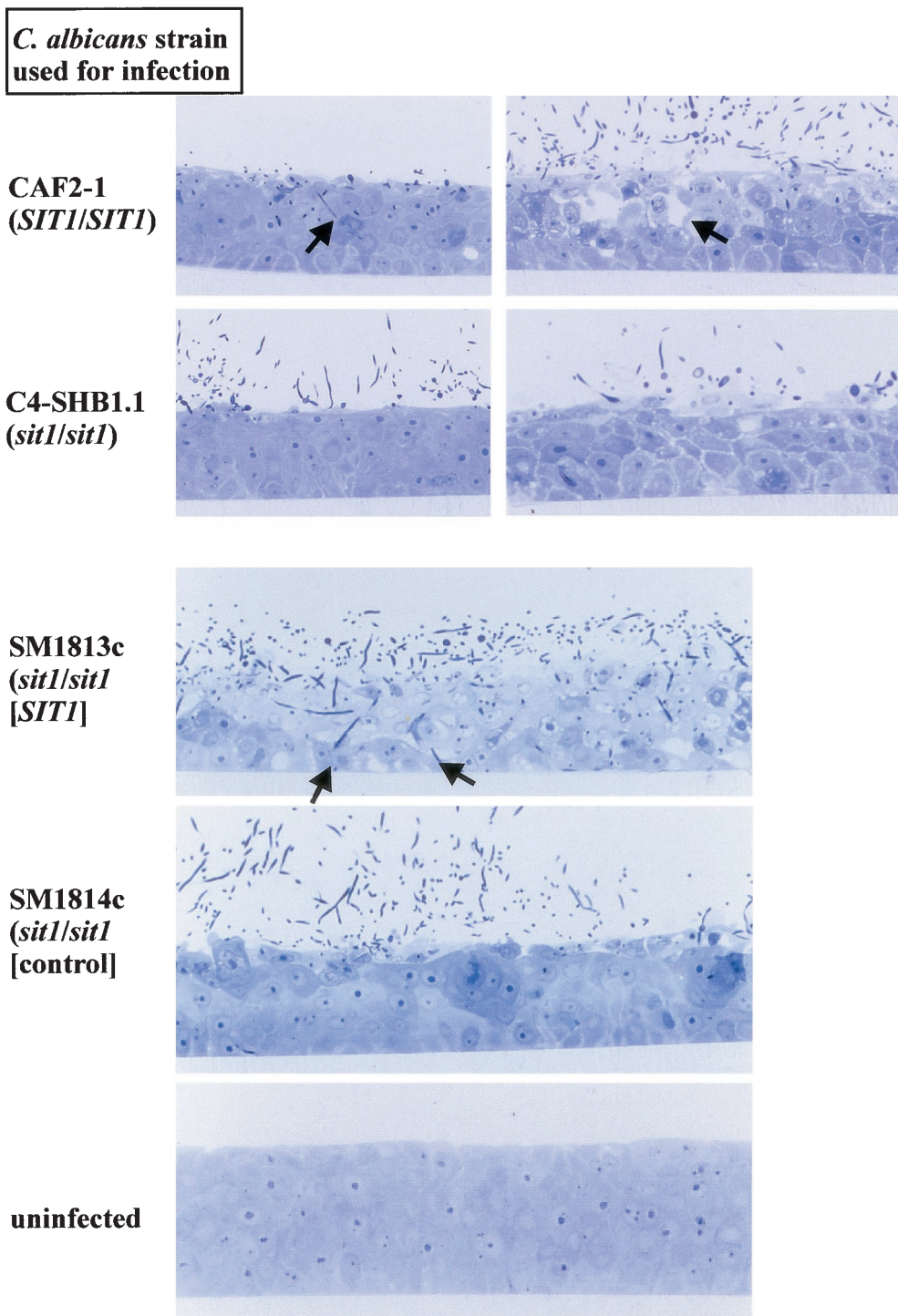[b] Growth zone diameters in agar diffusion tests: ++, 30 to 35 mm; +, 25 to 30 mm; +/−, up to 10 mm.

FIG. 4. Infection of an RHE model by 2×10^6 *C. albicans* cells. RHE infected by strains CAF2-1 and C4-SHB1.1 are shown at 6 and 22 h of incubation; RHE infected by strains SM1813c and SM1814c are shown at 12 h of incubation. Note the deep penetration and formation of vacuoles by *SIT1*+ strains (arrows).

defective in the uptake of CIT, while HEM utilization was not blocked in either the *sit1* or *ftr1* mutant.

The specificity of Sit1p for FCH-type siderophores was confirmed by the expression of Sit1p in the heterologous yeast *S. cerevisiae*. In agreement with the results of a recent study (1),

we found that an Sit1p-producing *S. cerevisiae* transformant lacking known components of reductive and siderophore-mediated iron uptake was able to utilize FCH and related siderophores, but not ferrioxamines, as sources of iron. Possibly, the lack of transport function of Sit1p, which was reported in
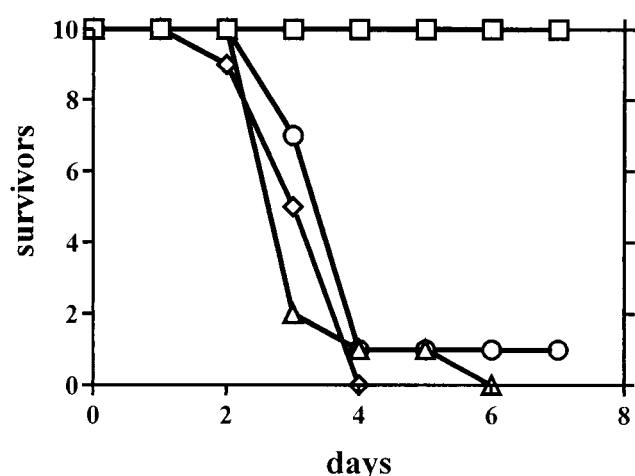
FIG. 5. Virulence of *C. albicans* in mice. *C. albicans* cells (10^6; yeast form) were injected into the tail veins of BALB/c mice ($n = 10$), and the survival of mice was monitored daily. The strains used were CAF2-1 (*SIT1/SIT1*) (△), C4-SHB1.1 (*sit1/sit1*) (◇), C4-SH1.1 (*SIT1/sit1*) (○), and C4-SHHB4/21-VII (*sit1/sit1/sit1*) (□).

another recent study (28), was due to accidental mutations in the expression plasmid or in the host. Thus, it appears that *C. albicans* has at least the following four uptake systems for iron chelates: (i) Sit1p, as an uptake system for FCH-type siderophores; (ii) a ferrioxamine uptake system including elements of the reductive iron import such as Ftr1p; (iii) conceivably, an uptake system for CIT, which also requires Ftr1p; and (iv) an uptake system for HEM, which does not depend on either Sit1p or Ftr1p.

The effect of the *sit1* mutation on virulence was tested (i) in vitro by using RHE, which is a model of human oral mucosa (39), and (ii) in the standard intravenous model of systemic infection in mice. In the mucosal model, *sit1* mutants were clearly compromised in invading keratinocyte layers and caused less damage than *SIT1* cells, which penetrated deeply and caused the formation of cavities within the epithelium. The mutant phenotype was absent in the *sit1/SIT1* heterozygous strain and was restored by reintegrating a single copy of *SIT1* at its genomic locus. On the other hand, the virulence of the *sit1* mutant in the systemic model of infection was comparable to that of the wild-type and reconstituted *SIT1* strains, indicating that siderophore transport is not of importance once the fungal pathogen is in the bloodstream. This assumption is supported by the pattern of iron chelate uptake in *C. albicans* discussed above, since HEM is a source of iron even in *sit1* and *ftr1* mutants. Presumably, the secretion of proteinases by *C. albicans*, which are able to degrade heme-containing proteins including hemoglobin (36), helps to liberate the heme moiety and to provide a source of iron; in addition, transferrin is likely to be another source of iron, permitting growth in blood and possibly within cells. Other examples of the differential effects of *C. albicans* mutations on infectivity in the mucosal and systemic models are known and indicate the importance of virulence traits that depend on specific host niches. For example, *phr2* mutants were virulent in the systemic mouse model of infection but were avirulent in a vaginal infection model; in the case of the *phr1* mutant, the phenotype was the inverse (7).

Phospholipase D1 and Sap4p to Sap6p were required for virulence in the systemic model but were not required in the mucosal model (19, 38, 40). Interestingly, during the course of *SIT1* disruption, we isolated a mutant containing three alleles of *sit1* (strain C4-SHHB4/21-VII) which with respect to siderophore utilization had the same phenotype as an *sit1/sit1* mutant. However, the three-allele mutant strain was completely avirulent in the systemic model of infection and showed reduced growth and defective hyphal morphogenesis. Thus, its virulence phenotype is not caused by a lack of Sit1p but is related to other unknown genetic alterations occurring in this strain. For example, it is known that the increased gene dosage of a repressor protein may prevent utilization of certain carbon sources in *C. albicans* (21). The three *sit1* alleles we have found are reminiscent of the *ftr1* mutant, which has also been reported as avirulent (33).

REFERENCES

1. **Ardon, O., H. Bussey, C. Philpott, D. McVey Ward, S. Davis-Kaplan, S. Verroneau, B. Jiang, and J. Kaplan.** 2002. Identification of a *Candida albicans* ferrichrome transporter and its characterization by expression in *Saccharomyces cerevisiae*. J. Biol. Chem. **276:**43049–43055.
2. **Askwith, C., D. Eide, A. van Ho, P. S. Bernhard, L. Li, S. Davis-Kaplan, D. M. Sipe, and J. Kaplan.** 1994. The *FET3* gene of *S. cerevisiae* encodes a multicopper oxidase required for ferrous iron uptake. Cell **76:**403–410.
3. **Askwith, C. C., D. de Silva, and J. Kaplan.** 1996. Molecular biology of iron acquisition in *Saccharomyces cerevisiae*. Mol. Microbiol. **20:**27–34.
4. **Blaiseau, P. L., E. Lesuisse, and J. M. Camadro.** 2001. Aft2p, a novel iron-regulated transcription activator that modulates, with Aft1p, intracellular iron use and resistance to oxidative stress in yeast. J. Biol. Chem. **276:**34221–34226.
5. **Bockmühl, D. P., S. Krishnamurthy, M. Gerads, A. Sonneborn, and J. F. Ernst.** 2001. Distinct and redundant roles of the two protein kinase A isoforms Tpk1p and Tpk2p in morphogenesis and growth of *Candida albicans*. Mol. Microbiol. **42:**1243–1257.
6. **Dancis, A., D. G. Roman, G. J. Anderson, A. G. Hinnebusch, and R. D. Klausner.** 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.
7. **De Bernardis, F., F. A. Mühlschlegel, A. Cassone, and W. A. Fonzi.** 1998. The pH of the host niche controls gene expression in and virulence of *Candida albicans*. Infect. Immun. **66:**3317–3325.
8. **Dix, D. R., J. T. Bridgham, M. A. Broderius, C. A. Byersdorfer, and D. J. Eide.** 1994. The *FET4* gene encodes the low affinity Fe(II) transport protein of *Saccharomyces cerevisiae*. J. Biol. Chem. **269:**26092–26099.
9. **Eck, R., S. Hundt, A. Härtl, E. Roemer, and W. Kuenkel.** 1999. A multicopper oxidase gene from *Candida albicans*: cloning, characterization and disruption. Microbiology **145:**2415–2422.
10. **Fonzi, W. A., and M. Y. Irwin.** 1993. Isogenic strain construction and gene mapping in *Candida albicans*. Genetics **134:**717–728.
11. **Georgatsou, E., and D. Alexandraki.** 1994. Two distinctly regulated genes are required for ferric reduction, the first step of iron uptake in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **14:**3065–3073.
12. **Gietz, R. D., and A. Sugino.** 1988. New yeast-*Escherichia coli* shuttle vectors constructed with *in vitro* mutagenized yeast genes lacking six-base pair restriction sites. Gene **74:**527–534.
13. **Gómez García, M., J.-E. O'Connor, L. Latorre García, S. I. Martínez, E. Herrero, and L. del Castillo Agudo.** 2001. Isolation of a *Candida albicans* gene, tightly linked to *URA3*, coding for a putative transcription factor that suppresses a *Saccharomyces cerevisiae aft1* mutation. Yeast **18:**301–311.
14. **Güldener, U., S. Heck, T. Fiedler, J. Beinhauer, and J. H. Hegemann.** 1996. A new efficient gene disruption cassette for repeated use in budding yeast. Nucleic Acids Res. **24:**2519–2524.
15. **Heymann, P., J. F. Ernst, and G. Winkelmann.** 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.

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

17. **Heymann, P., J. F. Ernst, and G. Winkelmann.** 2000. A gene of the major facilitator superfamily encodes a transporter for enterobactin (Enb1) in *Saccharomyces cerevisiae*. Biometals **13:**65–72.

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

19. **Hube, B., D. Hess, C. A. Baker, M. Schaller, W. Schäfer, and J. W. Dolan.** 2001. The role and relevance of phospholipase D1 during growth and dimorphism of *Candida albicans*. Microbiology **147:**879–889.

20. **Ismail, A., G. W. Bedell, and D. M. Lupan.** 1985. Siderophore production by the pathogenic yeast, Candida albicans. Biochem. Biophys. Res. Commun. **130:**885–891.

21. **Janbon, G., F. Sherman, and E. Rustchenko.** 1998. Monosomy of a specific chromosome determines L-sorbose utilization: a novel regulatory mechanism in *Candida albicans*. Proc. Natl. Acad. Sci. USA **95:**5150–5155.

22. **Johnston, M., and R. W. Davis.** 1984. Sequences that regulate the divergent *GAL1-GAL10* promoter in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **4:**1440–1448.

23. **Knight, S. A. B., E. Lesuisse, R. Stearman, R. D. Klausner, and A. Dancis.** 2002. Reductive iron uptake by *Candida albicans*: role of copper, iron and the *TUP1* regulator. Microbiology **148:**29–40.

24. **Lee, K. L., H. R. Buckley, and C. C. Campbell.** 1975. An amino acid liquid synthetic medium for the development of mycelial and yeast forms of Candida albicans. Sabouraudia **13:**148–153.

25. **Leong, S., and G. Winkelmann.** 1998. Molecular biology of iron transport in fungi, p. 147–186. *In* A. Sigel and H. Sigel (ed.), Metal ions in biological systems, vol. 35. Marcel Dekker Inc., New York, N.Y.

26. **Lesuisse, E., M. Simon-Casteras, and P. Labbe.** 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.

27. **Lesuisse, E., P.-L. Blaiseau, A. Dancis, and J.-M. Camadro.** 2001. Siderophore uptake and use by the yeast *Saccharomyces cerevisiae*. Microbiology **147:**289–298.

28. **Lesuisse, E., S. A. B. Knight, J.-M. Camadro, and A. Dancis.** 2002. Siderophore uptake by *Candida albicans*: effect of serum treatment and comparison with *Saccharomyces cerevisiae*. Yeast **19:**329–340.

29. **Losberger, C., and J. F. Ernst.** 1989. Sequence and transcript analysis of the *C. albicans* gene (*URA3*) encoding orotidine-5′-phosphate decarboxylase. Curr. Genet. **16:**153–157.

30. **Minnick, A. A., L. E. Eizember, J. A. McKee, E. K. Dolence, and M. J. Miller.** 1991. Bioassay for siderophore utilization by *Candida albicans*. Anal. Biochem. **194:**223–229.

31. **Morrissey, J. A., P. H. Williams, and A. M. Cashmore.** 1996. *Candida albicans* has a cell-associated ferric-reductase activity which is regulated in response to levels of iron and copper. Microbiology **142:**485–492.

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

33. **Ramanan, N., and Y. Wang.** 2000. A high-affinity iron permease essential for *Candida albicans*. Science **288:**1062–1064.

34. **Ratledge, C., and L. G. Dover.** 2000. Iron metabolism in pathogenic bacteria. Annu. Rev. Microbiol. **54:**881–941.

35. **Robertson, L. S., H. C. Causton, R. A. Young, and G. R. Fink.** 2000. The yeast A kinases differentially regulate iron uptake and respiratory functions. Proc. Natl. Acad. Sci. USA **97:**5984–5988.

36. **Rüchel, R., R. Tegeler, and M. A. Trost.** 1982. Comparison of secretory proteinases from different strains of Candida albicans. Sabouraudia **20:**233–244.

37. **Rupniak, H. T., C. Rowlatt, E. B. Lane, J. G. Steele, L. K. Trejdosiewicz, B. Laskiewicz, S. Povey, and B. T. Hill.** 1985. Characteristics of four new human cell lines derived from squamous cell carcinomas of the head and neck. JNCI **75:**621–635.

38. **Sanglard, D., B. Hube, M. Monod, F. C. Odds, and N. A. Gow.** 1997. A triple deletion of the secreted aspartyl proteinase genes *SAP4*, *SAP5*, and *SAP6* of *Candida albicans* causes attenuated virulence. Infect. Immun. **65:**3539–3546.

39. **Schaller, M., W. Schäfer, H. C. Korting, and B. Hube.** 1998. Differential expression of secreted aspartyl proteinases in a model of human oral candidosis and in patient samples from the oral cavity. Mol. Microbiol. **29:**605–615.

40. **Schaller, M., H. C. Korting, W. Schäfer, J. Bastert, W. C. Chen, and B. Hube.** 1999. Secreted aspartic proteinase (Sap) activity contributes to tissue damage in a model of human oral candidosis. Mol. Microbiol. **34:**169–180.

41. **Sherman, F., G. R. Fink, and J. B. Hicks.** 1986. Methods in yeast genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

42. **Stearman, R., D. S. Yuan, Y. Yamaguchi-Iwai, R. D. Klausner, and A. Dancis.** 1996. A permease-oxidase complex involved in high-affinity iron uptake in yeast. Science **271:**1552–1557.

43. **Sweet, S. P., and L. J. Douglas.** 1991. Effect of iron concentration on siderophore synthesis and pigment production by *Candida albicans*. FEMS Microbiol. Lett. **80:**87–92.

44. **Weinberg, E. D.** 1999. The role of iron in protozoan and fungal infectious diseases. J. Eukaryot. Microbiol. **46:**231–238.

45. **Winkelmann, G., and H. Drechsel.** 1997. Microbial siderophores, p. 200–246. *In* H.-J. Rehm and P. Stadlers (ed.), Bio/Technology, 2nd ed. VCH, Weinheim, Germany.

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

47. **Yun, C.-W., T. Ferea, J. Rashford, O. Ardon, P. Brown, D. Botstein, J. Kaplan, and C. C. Philpott.** 2000. Desferrioxamine-mediated iron uptake in *Saccharomyces cerevisiae*. Evidence for two pathways of iron uptake. J. Biol. Chem. **275:**10709–10715.

48. **Yun, C.-W., J. S. Tiedeman, R. E. Moore, and C. C. Philpott.** 2000. Siderophore-iron uptake in *Saccharomyces cerevisiae*. J. Biol. Chem. **275:**16354–16359.