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Candida albicans Can Utilize Siderophore during Candidastasis Caused by Apotransferrin

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Ability of iron acquisition of pathogenic microorganisms functions as a virulence factor. *Candida albicans*, a fungal pathogen that requires iron for growth, is susceptible to growth retardation by high-affinity iron binding proteins such as transferrin. Recently, we reported that *C. albicans* could utilize the heme as a part of heme-containing proteins dissociated by heme oxygenase, CaHMX1. In search of another pathway that *C. albicans* can use to bypass the growth regulation produced by iron limitation, this present study examined utilization of non-candidal siderophores such as Desferal and rhodotorulic acid (RA) for acquisition of inorganic iron by the fungus. *C. albicans* secreting no siderophores was cultured in iron-free (pretreated with apotransferrin for 24 h) (culture medium). Once growth of the yeast reached stasis from iron starvation, a siderophore was added to the culture media. Results showed that cultures containing apotransferrin within a dialysis membrane recovered growth to the level of untreated controls, whereas *C. albicans* yeast cells in direct contact with soluble iron-free (apo) transferrin recovered growth only partially. When static growth from iron limitation was reached, the addition of siderophore-apotransferrin complex to culture medium also permitted the yeast to recover growth from apotransferrin growth regulation. All the data show that *C. albicans* can utilize the non-candidal siderophores for iron acquisition under transferrin regulation as can pathogenic bacteria.

Key words: *Candida albicans*, Iron, Transferrins, Sideophore, Desferal, Rhodotorulic acid

INTRODUCTION

In mammalian body fluids such as serum, high affinity iron-binding proteins like principally transferrin and lactoferrin ensure that free ionic iron is unavailable to invading microorganisms (Weinburg, 1971; Bullen, 1981; Griffiths, 1985). Consequently, to multiply successfully *in vivo*, pathogenic microbes must possess mechanisms for utilizing the iron from host iron-binding proteins, or develop some alternative means for acquiring iron from other organic iron-binding proteins. One basic type of high-affinity iron uptake system capable of removing iron from iron-binding proteins is mediated by a low molecular weight iron chelator called a siderophore.

Many bacteria secrete siderophores (Griffiths, 1983; Neilands, 1981; Perry and Brubaker, 1979; Brock and Ng, 1983) during conditions of iron restriction *in vitro*. Also certain fungi, including *Ustilago sphaerogena* (Emery, 1971), *Neurospora crassa* (Muller and Winklemann, 1981), *Rhodotorula pilimanae* (Atkin and Neilands, 1968), and the pathogenic fungus, *Histoplasma capsulatum* (Burt *et al.*, 1981), have reported to produce siderophores for iron acquisition for their growth when cultured under conditions of iron restriction. In addition to using autologous siderophores, certain microbes utilize heterologous (made by other species of microbes) siderophores to support their growth. For example, some strains of enteric bacteria utilize, interchangeably, siderophores from different bacteria (Miles and Khimji, 1975). Moreover, *E. coli* and *Salmonella typhimurium* are known to utilize fungal siderophores, such as ferrichrome, to support their growth (Leong and Neilands, 1976; Luckey, *et al.*, 1972). Also, *Streptomyces pilomanae* that produces deferoxamine (Desferal) can assimilate iron by using the heterologous siderophore, rhodotorulic acid, produced by *Rhodotorula pilimanae* (Muller *et al.*, 1984). Thus, microorganisms can use not only siderophores produced by members of common

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species, but also use the siderophores of unrelated species. From the perspective of microbial pathogenesis, it has been shown that some pathogenic microbes can utilize siderophores to establish a successful infection in the host under iron-restricted conditions (Weinberg, 1971, 1975; Neilands, 1982; Payne and Flinkelstein, 1978; Griffiths and Humphreys, 1980). These data indicate that siderophores can play an active role in bypassing the growth regulatory effects of the transferrin as a natural host resistance.

In case of *Candida albicans*, it has been controversial until now that the fungus can secreted both hydroxamate and phenolate-type siderophores when grown under iron-restricted conditions (Ismail and Lupan, 1986). Recently, Heymann *et al.,* reported that *C. albicans* contained a close homologue of yeast siderophore transporters, Sit1p/Arn1p (Heymann *et al.*, 2002). In contrast, other study describes the genes mediating uptake and utilization of iron from siderophores in *C. albicans* is not identified (Lesuisse *et al.*, 2002).

Furthermore, how the fungus survives and initiates infections within the host under the iron regulation by tranferrins remains unclear although it has been a long time that the transferrins are inhibitors of candidal growth. The yeast must evolve, of necessity, a means for circumventing the iron-deprivation effect of the transferrins. For part of this, we have recently reported that *C. albicans* utilizes heme part of the ferroproteins under the iron-deficient condition caused by the transferrin (Han *et al.*, 2005c). In addition, other's recent data indicate that *C. albicans* can obtain iron acquisition from transferrin by a reductive pathway *via* FTR1 (Knight *et al.*, 2005). Based on all these aspects, we investigated to determine if *C. albicans* can utilize heterlogous siderophores as a means of obtaining iron to bypass transferrin-mediated growth regulation.

MATERIALS AND METHODS

Reagents

Iron-free (apo) transferrin (human) was purchased from Sigma (St. Louis, MO). Desferal and rhodotorulic acid were obtained from CIBA Pharmaceutical Co., Summit, NJ. Dialysis bag [6~8000 daltons (Da) mol. wt. cut-off] was obtained from Spectrum Medical Industries, Inc., (Los Angeles, CA).

Organism and culture media

C. albicans 3153A and 8011 that were obtained from the Mycological Reference Laboratories, Public Health Laboratoty Service, U.S.A. and CA-1 strain, which was previously characterized (Han *et al.*, 2000, 2001; Han and Lee, 2005a; Han, 2005b), were used. The strains were all maintained on Sabouraud dextrose agar (SDA; Difco Laboratories, Detroit, MI) slants and cloned on the same fresh medium every month to ensure culture homogeneity. Inocula were prepared from overnight cultures in a Sabouraud dextrose broth at 37°C. Experiments with the yeast cells were carried out in a modified Winge (Garcia-Mendoza and Novales-Lindieu, 1968) liquid medium (pH 7.5; 0.3% yeast extract, 0.2% glucose) supplemented with 15 mM $NaHCO_3$, which was sterilized by filtration with a disposable filter apparatus (pore size = 0.45 μM; Nalge Company, Rochester, NY) as previously described (Han, 2005c). The addition of $NaHCO_3$ was to enhance antifungal activity of apotransferrin (Phelps and Antonini, 1975; Graham and Bates, 1976).

Detection of siderophore production

To determine if the *C. albicans* strains secrete siderophore, the strains were cultured in iron-deficient Winge liquid culture medium that was made by placing iron-free apotransferrin (2 mg/mL; Sigma, St. Loius, MO) with a dialysis bag in the culture medium as previously described (Han, 2005c). The culture fluids were collected at various time points and assayed for siderophore production with use of chromo azurol S medium (CAS plate: Difco) (Schwyn and Neilands, 1987). Before detection on the CAS plates, the culture supernatants were concentrated by lyophilization (FD-1000, Eyela, Japan). As positive controls for the assay, known siderophore standards such as Desferal and rhodotorulic acid (RA) were used. In experiments, 10 mL of each culture supernatant were dropped on a surface of green-colored CAS plate. Color change from the green to yellow indicates presence of siderophore according to the manufacturer's guideline.

Siderophore utilization assay

Desferal and RA were used to determine the ability of *C. albicans* to utilize siderophores to bypass the growth regulation mediated by apotransferrin. The iron affinity (Log Ka) of these siderophores is 30.6 and 31.1 corresponding to Desferal and RA, respectively (Anderegg *et al.*, 1963; Gentry *et al.*, 1986). These iron affinities are greater than the transferrins whose affinities are 20 (lactoferrin; Edward *et al.*, 1987; Aisen and Listowsky, 1980), 23.6 (serum transferrin; Carrano and Raymond, 1979) and 29 (ovotransferrin; Waner and Weber, 1953). In this work, serum transferrin was used. In experiments, the Desferal and RA were dissolved in Winge liquid medium at 2.3, 23, or 230 μM and were added to the culture medium. By calculation, the concentrations corresponded to siderophore to transferrin ratios of 0.1:1, 1:1, and 10:1, respectively. The iron-limited Winge medium was prepared by pre-treating it with apotransferrin that was contained in a dialysis bag as described above for 24 h before inoculation with *C. albicans* (5×10^5 yeast cells/mL). This

preparation was referred as indirect contact. The culture was incubated at 37°C for 24 h. At designated time periods, the cell number was determined by hemocytometer.

In another experiment, *C. albicans* was inoculated into Winge culture media containing apotransferrin, and the culture was incubated under the same cultural condition as described above. This preparation was referred as direct contact hereafter.

Prior to those experiments, effects of Desferal and RA on *C. albicans* growth were determined in Winge liquid culture medium in the absence of the apotransferrin.

Utilization assay of apotransferring-siderophore

To confirm the nature of *C. albicans*-apotransferrin-siderophore interaction, iron-Desferal complex (Fe-DF) was prepared at a molar ratio of 3 : 4 to ensure the availability of free ionic iron. This preparation was tested by the indirect and the direct methods as mentioned above. In these experiments, inoculation of the yeast was identical as described the above. In another experiment, iron-RA complex was tested in the identical way as the above.

Statistical anaylsis

The results are expressed as mean ± standard error. The significance of difference among the groups was determined by Student *t*-test. *P* values less than 0.05 were considered statistically significant.

RESULTS

Detection of siderophore from *C. albicans* strains

None of the *C. albicans* strains were able to produce siderophores on CAS plate, even after the culture supernatants were concentrated ten fold by lyophilization, whereas known siderophore standards, Desferal and RA used as positive controls consistently yielded positive results for siderophore on the CAS plates (Table I). Based on these results, *C. albicans* 3153A was inclusively used in the following experiments.

Table I. None of the *C.albicans* strains produce any siderophore as determined with CAS plate

	Color change
Supernatant of	
3153A	–*1
8011	–
CA-1	–
Siderophore of	
Desferal	+*2
RA*3	+

Note:
*1No siderophore production
*2Siderophore production
*3RA stands for rhodotorulic acid.

Utilization of siderophore by *C. albicans* 3153A during apotransferrin-mediated growth regulation

Results showed Desferal at the higher concentrations such as 23 μM and 230 μM was found to be inhibitory to growth of *C. albicans* yeast cells in the absence of apotransferrin (Fig. 1). A similar phenomenon was also observed when Desferal, and the yeast cells were inoculated simultaneously into the pretreated culture medium by apotransferrin. The addition of Desferal resulted in initial inhibition rather than supported candidal growth (Figs. 2A & B). As shown in Fig. 1, the magnitude of inhibition was related to siderophore concentration, particularly for yeast cells cultured by the indirect method (Fig. 2A), not in contact with the apo-protein (Fig. 2B). However, as the incubation progressed, the initial inhibition diminished permitting growth recovery from the growth regulation by apotransferrin, regardless of whether the apotransferrin was in contact with the yeast cell. A concentration of 2.3 μM produced a full recovery of growth in the medium treated by the indirect method (Fig. 2A), but only partial recovery was observed when the apo-protein remained in contact with yeast cells (Fig. 2B). The differences between experimental and control cultures were statistically significant ($P < 0.05$). Because yeast cells treated by the indirect method recovered growth fully, the interaction of apotransferrin in the direct contact medium must have altered the response to Desferal. In other experiments, Desferal was added at time 48 h – the point when the growth stasis occurred following apotransferrin regulation. In this case, Desferal stimulated growth

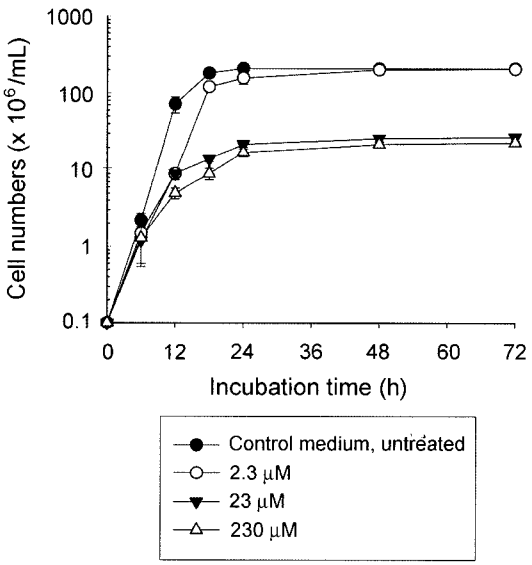

Fig. 1. Dose-dependent growth inhibition of *C. albicans* by iron-free Desferal in Winge liquid culture medium. Yeast cells (10^5 cells/mL) were inoculated with DF into the culture. Cell counts were determined by microscopy. Values were expressed as mean ± S.E. of quadruplicate cultures.

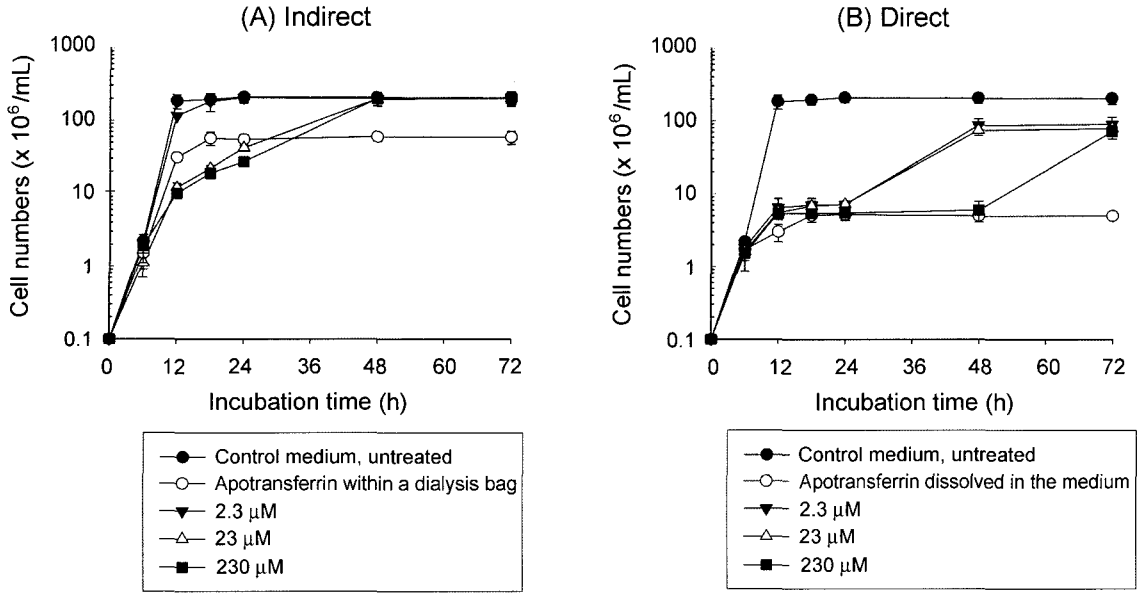

Fig. 2. Effect of iron-free Desferal on *C. albicans* growth during candidastatic growth regulation by apotransferrin: simultaneous addition of siderophore and yeast cell inoculum. Winge liquid culture medium was pretreated with apotransferrin (2 mg/mL) for 24 h either the indirect contact or the direct contact method. Yeast cells (10^5 cells/ml) were inoculated simultaneously (T=0 h) with DF at 0, 2.3, 23, and 230 μM, respectively into the culture. Cell counts were determined by microscopy. Values were expressed as mean ± S.E. of quadruplicate cultures.
Panels: A) indirect contact treatment: control, apotransferrin within a dialysis bag; B) direct contact treatment: control, apotransferrin in the culture.

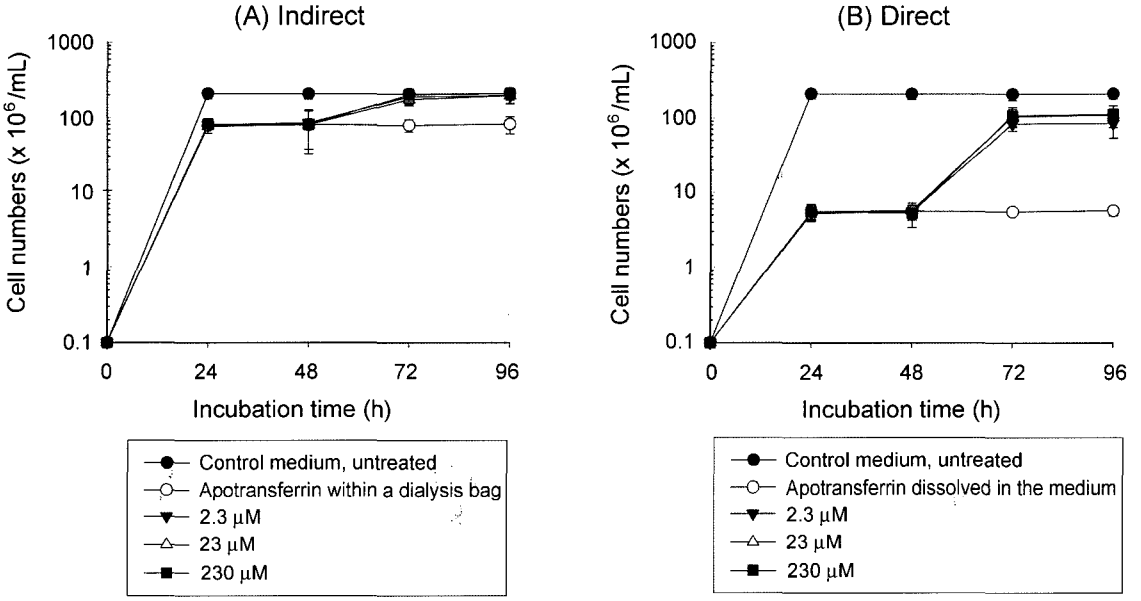

Fig. 3. Effect of iron-free Desferal on *C. albicans* growth during candidastatic growth regulation by apotransferrin: siderophore added 48 h after yeast cell inoculation. Winge liquid culture medium was pretreated with apotransferrin (2 mg/mL) for 24 h by either the indirect contact or the direct contact method. The inoculum was 10^5 cells/mL. The siderophores were added when growth reached stasis (T=48 h) from the effects of iron limitation. Cell counts were determined by microscopy. Values were expressed as mean ± S.E. of quadruplicate cultures.
Panels: A) indirect contact treatment; control, apotransferrin within a dialysis bag: B) direct contact treatment: control, apotransferrin in the culture.

without any period of inhibition (Figs. 3A & B). Once again, in yeast cells in the medium treated by the indirect method (Fig. 3A) fully recovered growth to the level of untreated controls. Differences between the two cultures were statistically significant ($P < 0.05$). In contrast, yeast cells cultured in contact with apotransferrin (Fig. 3B) only partially recovered growth.

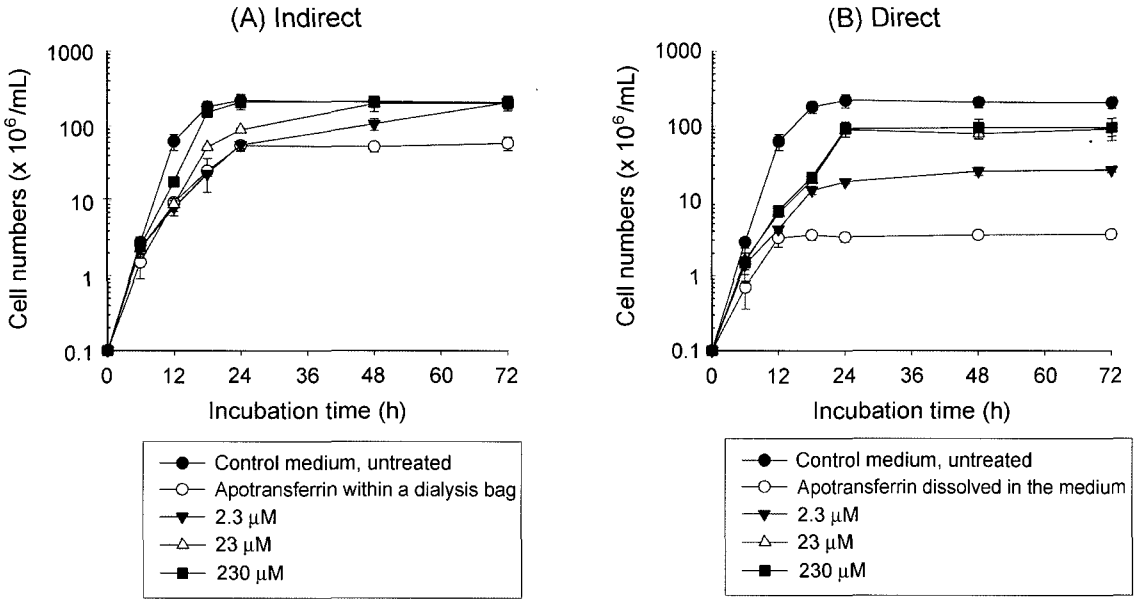

Fig. 4. Effect of iron-laden Desferal (Fe-DF) on *C. albicans* growth during candidastatic growth regulation by apotransferrin: Simultaneous addition of Fe-siderophore and yeast cell inoculation. Winge liquid culture medium was pretreated with apotransferrin (2 mg/mL) for 24 h either the indirect contact or the direct contact method. Yeast cells (10^5 cells/ml) were inoculated simultaneously (T=0 h) with Fe-DF at 2.3, 23, and 230 μM into the culture. The Desferal was pretreated with ferric chloride at a molar ratio of 4:3. Cell counts were determined by microscopy. Values were expressed as mean ± S.E. of quadruplicate cultures.
Panels: A) indirect contact treatment; control, apotransferrin within a dialysis bag: B) direct contact treatment: control, apotransferrin in the culture.

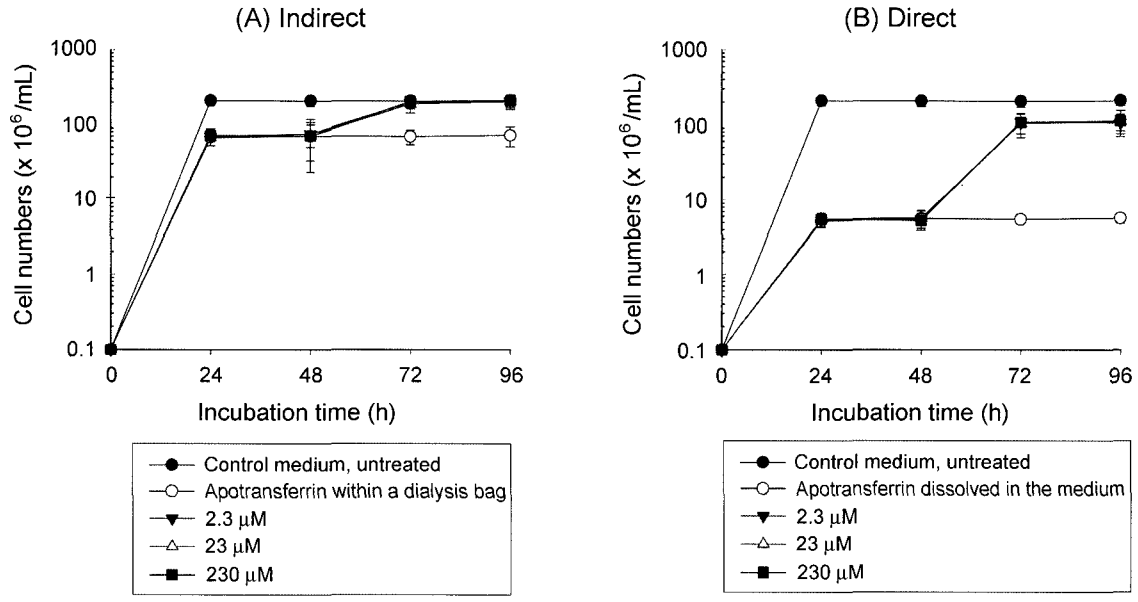

Fig. 5. Effect of iron-laden Desferal (Fe-DF) on *C. albicans* growth during candidastatic growth regulation by apotransferrin: Fe-siderophore added 48 h after yeast cell inoculation. Winge liquid culture medium was pretreated with apotransferrin (2 mg/mL) for 24 h either the indirect contact or the direct contact method. The inoculum was 10^5 cells/ml. The Fe-siderophore was added when growth reached stasis (T=48 h) from the effect of iron limitation. The Desferal was pretreated with ferric chloride at a molar ratio of 4:3. Cell counts were determined by microscopy. Values were expressed as mean ± S.E. of quadruplicate cultures.
Panels: A) indirect contact treatment; control, apotransferrin within a dialysis bag: B) direct contact treatment: control, apotransferrin in the culture.

Utilization of apotransferrin-siderophore complex by *C. albicans*

When the Fe-DF complex was added with simultaneous (T=0 h) inoculation of yeast to the culture, growth stimulation was enhanced (Figs. 4A & B). This effect was a dose-dependent. The elevated concentrations of Fe-DF served

to produce a more rapid recovery from the regulation of iron limitation. Cultures containing apotransferrin within a dialysis bag recovered growth to the level of untreated controls. Those yeast cells in contact with soluble apo-protein recovered growth only partially. When static growth from iron limitation was reached (T=48 h), the addition of Fe-DF to the culture also permitted the yeast to recover growth from the growth regulation (Figs. 5A & B). In this experiment, growth stimulation was enhanced at the same rate, regardless of the concentration of Fe-DF added to the culture. In addition, the results showed the lowest concentration (2.3 μM) of Fe-DF was sufficient to stimulate a maximal recovery of growth. The addition of surplus Fe-DF (23-230 μM) did little to aid recovery. In addition, treatment with Fe-RA resulted in the same phenomenon as did with Fe-Desferal (data not shown).

DISCUSSION

A major role of the transferrin in host defense is to chelate free iron, thereby making it unavailable to invading pathogens. While reviewing the results from our study, it became obvious that both Desferal and RA may act in several different roles during the *C. albicans* growth. When no other iron chelator is present, the siderophores alone can be a growth inhibitor. This phenomenon is concentration dependent because 2.3 μM had no effect on growth, but 23-230 μM was inhibitory. Possibly, in the absence of plentiful iron, or in the presence of limited iron as found within a culture, Desferal and RA, respectively, at elevated concentrations becomes an iron acquisition antagonist. In this role, the siderophores chelate not only extracellular iron but also intracellular iron, thereby decreasing the iron available for metabolism. In contrast, when yeast cell growth is static from iron limitation by apotransferrin, Desferal and RA act as a growth agonist, probably by providing iron purloined from the apo-protein. In this instance, disruption of intracellular iron pools does not occur because the iron has already been diluted to a critical concentration during cell multiplication.

The concentration of siderophore is not as crucial to recovery. Because once the minimal quantity has been obtained from the apotransferrin, growth ensues regardless of the concentration of remaining unsaturated siderophore. What then happens to growth when two iron chelators (siderophore-apotransferrin) are added into culture medium, the results are neither easily explained nor readily understood. Possible explanations for this as follow; first of all, the addition of Desferal (or RA) and apotransferrin is not simultaneous. The apo-protein has been in the medium for 24 h before the addition of yeast and Desferal, thereby permitting the apo-protein to initially chelate most available iron. In the absence of apotransferrin-yeast interaction, a low concentration of Desferal is stimulatory, probably by taking iron from the apo-protein and providing it to the yeast. With elevated concentrations (23-230 μM) of Desferal (or RA), an initial period of growth retardation is followed by total recovery. This result is paradoxical because Desferal alone at these concentrations was a growth inhibitor. Similarly, yeast cells in contact with apotransferrin undergo an identical growth response with an initial period of the retardation followed by recovery. The interchange of iron during yeast cell-apotransferrin-siderophore interaction is important to ensure yeast multiplication. From this analysis, it became apparent that the level of iron-siderophore saturation may provide some understanding as to the nature of the interaction. However, determination of possible mechanisms for that is beyond the scope of this study.

In summary, these studies have shown that *C. albicans* is like a number of other pathogen in which it can utilize heterologous (non-candidal) siderophores to circumvent the growth inhibitory effects of the transferrin.

REFERENCES

Aisen, P. and Listowsky, I. A., Iron transport and storage protein. *Ann. Rev. Biochem.*, 49, 357-393 (1980).

Anderegg, G., L'Eplattenier, F. L., and Schwarzenbach, G., Hydroxamate complexes. II. An application of the pH method. *Helv. Chim. Acta.*, 46, 1400-1408 (1963).

Atkin, C. L. and Neilands, J. B., Rhodotorulic acid, a diketopiperazine dihydroxamic acid with growth-factor activity. I. Isolation and characterization. *Biochemistry*, 7, 3734-3739 (1968).

Brock, J. H. and Ng, J., The effect of desferrioxamine on the growth of *Staphylococcus aureus, Yersinia enterocolitica, and Streptococcus faecalis* in human serum: uptake of desferrioxamine-bound iron. *FEMS Microbiol. Lett.*, 20, 439-442 (1983).

Bullen, J. J., The significance of iron in infection. *Rev. Infec. Dis.*, 3, 1127-1138 (1981).

Burt, W. R., Underwood, A. L., and Appleton, G. L., Hydroxamic acid from *Histoplasma capsulatum* that displays growth factor activity. *Appl. Enviro. Microbial.*, 42, 560-563 (1981).

Carrano, C. J. and Raymond, K. N., Ferric iron sequestering agents. 2. Kinetics and mechanism of iron removal from transferring by enterobactin and synthetic tricatechols. *J. Am. Chem. Soc.*, 101, 5401-5404 (1979).

Edward, N. B., Rumbell, S. V., and Anderson, B. F., Transferrin: insight into structure and function from studies on lactoferrin. *TIBS.*, 12, 350-354 (1987).

Emery, T., Role of ferrichrome as a ferric ionophore in Ustilago sphaerogena. *Biochemistry*, 10, 1483-1488 (1971).

Garcia-Mendoza, C. and Novales-Lidieu, M., Chitin in the new wall of regenerating protoplasts of *Candida utilis*. *Nature*,

220, 1035-1036 (1968).

Gentry, M. J., Confer, A. W., Weinberg, E. D., and Homer, J. T., Cytoxin (leukotoxin) production by Pasteurella Haemolytica: Requirement for as iron-containing compound. *Am. J. Vet. Res.*, 47, 1919-1923 (1986).

Graham, G. F. and Bates, G. W., Approaches to the standardization of serum unsaturated iron-binding capacity. *J. Lab. Clin. Med.*, 88, 477-486 (1976).

Griffiths, E. and Humphreys., Isolation of enterochelin from the peritoneal washings of guinea pigs lethally infected with *Escherichia coli*. *Infect. Immun.*, 28, 286-289 (1980).

Griffiths, E., Availability of iron and survival of bacteria in infection. *In* Medical Microbiol., (Eds. Easmon, C. S. F. *et al.*). Vol. 3, pp 153-157. *Academic press*, London (1983).

Griffiths, E. Candidate virulence markers. *In*: The virulence of *Escherichia coli* (Ed. Sussman, M.). *Academic Press, London*, pp. 193-226 (1985).

Han, Y., Riesselman, M. H., and Cutler, J. E., Protection against candidiasis by an immunoglobulin G3 monoclonal antibody specific for the same mannotriose as an IgM protective antibody. *Infect. Immun.*, 68, 1649-1654 (2000).

Han, Y., Kozel, T. R., Zhang, M. X., MacGill, R. S., Caroll, M. C., and Cutler, J. E., Complement is essential for protection by an IgM and an IgG3 monoclonal antibody against experimental, hematogenously disseminated candidiasis. *J. Immunol.*, 167, 1550-1557 (2001).

Han, Y. and Lee, J., Berberine synergy with amphotericin B against disseminated candidiasis. *Biol. Pharm. Bull.*, 28, 541-544 (2005a).

Han, Y., Ginkgo terpene component has an anti-inflammatory effect on *Candida albicans*-caused arthritic inflammation. *Int. Immunopharm.*, 5, 1049-1056 (2005b).

Han, Y., Utilization of ferroprotein by Candida albicans during candidastasis by apotransferrin. *Arch. Pharm. Res.*, 28, 963-969 (2005c).

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

Ismail, A. and Lupan, D. M., Utilization of siderophores by *Candida albicans*. *Mycopathologia*, 96, 109-113 (1986).

Knight, S. A., Vilaire, G., Lesuisse, E., and Dancis, A., Iron acquisition by *Candida albicans* depends on the reductive pathway. *Infect. Immun.*, 73, 5482-5492 (2005).

Leong, M. and Neilands, J. B., Mechanisms of siderophore iron transport in enteric bacteria. *J. Bacteriol.*, 126, 823-830 (1976).

Lesuisse, E., Knight, S. A., Camadro, J. M., and Dancis, A., Siderophore uptake by *Candida albicans*: effect of serum treatment and comparison with *Saccharomyces cerevisiae*. *Yeast.*, 15, 329-340 (2002).

Luckey, M., Pollack, J. R., Wayne, R., Ames, B. N., and Neilands, J. B., Iron uptake in *Salmonella typhimurium*: Utilization of exogenous siderochromes as iron carriers. *J. Bacteriol.*, 11, 731-738 (1972).

Miles, A. A. and Khimji, P. L., Enterobacterial chelators of iron: Their occurrence, detection, and relation to pathogenicity. *J. Med. Microbial.*, 8, 477-490 (1975).

Muller, G. and Winklemann, G., Binding of siderophores to isolated plasma membranes of *Neurospora crassa*. *FEMS Microbiol. Lett.*, 10, 327-331 (1981).

Muller, G., Matzanke, F. M. B. F., and Raymond, K. N., Iron transport in *Streptomyces pilosus* mediated by ferrichrome, rhodotorulic acid, and enantiorhodotorulic acid. *J. Bacteriol.*, 160, 313-318 (1984).

Neilands, J. B., Iron absorption and transport in microorganism. *Ann. Rev. Nutr.*, 1, 27-46 (1981).

Neilands, J. B., Microbial envelope proteins related to iron. *Ann. Rev. Microbial.*, 36, 285-309 (1982).

Payne, S. M. and Flinkelstein, R. N., Critical role of iron in host-baxterial interaction. *J. Clin. Invest.*, 61, 1428-1440 (1978).

Perry, R. D. and Brubaker, R. R., Accumulation of iron by *Yersiniae*. *J. Bacteriol.*, 137, 1290-1298 (1979).

Phelps, C. F. and Antonini, E., A study of the kinetics of iron and copper binding to hen ovotrasferrin. *J. Biochem.*, 147, 385-391 (1975).

Schwyn, B. and Neilands, J. B., Universal chemical assay for the detection and determination of siderophores. *Anal. Biochem.*, 160, 47-56 (1987).

Waner, R. C. and Weber, I., The metal combining properties of conalbumin. *J. Am. Chem. Soc.*, 75, 5094-5101 (1953).

Weinberg, E. D., Nutritional immunity. *J. Am. Med. Assoc.*, 231, 39-41 (1975).