Heterologous expression of Pce1 in Aspergillus niger inhibits enzymatic fermentation of ferric iron

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Abstract

The fungus Paracoccidioides brasiliensis, the etiological agent of paracoccidioidomycosis (PCM), is a very important fungal pathogen. It was initially reported that P. brasiliensis was able to penetrate into host tissues through host-specific barriers and to take up iron from ferrioxamine ferrea (Foxf1), the only metal ion available to infect Paracoccidioides brasiliensis. However, this observation was not true for all other iron-regulated fungi including Paracoccidioides brasiliensis, Aspergillus niger and Candida albicans. Similarly, the ability of P. brasiliensis to uptake and internally store iron in host tissues was not true of any of the host iron uptake mechanisms tested. Similarly, the depletion of iron in host tissues by P. brasiliensis did not result in an alteration of the transcriptomic profile of the infected lung tissue. However, the transcriptomic profile changes caused by depletion of iron in the lung, in peripheral tissues and in the serum of infected mice were significantly greater in P. brasiliensis compared to other fungi. In conclusion, our data from these studies shed new light on the specific mechanisms of Paracoccidioides brasiliensis towards host iron uptake mechanisms.

Introduction

Iron overload could be a possible cause for S. aureus biofilm formation and the stress of biofilm formation (Rohwer and van der Werf 2002). Iron overload was also reported in C. albicans (Ikeda et al. 1998). In the case of iron-starved cells, iron availability was increased by manipulating the expression of iron permease, Ftr1, in response to iron starvation (Ikeda et al. 1998). In S. aureus, disruption of the gene encoding the copper transporter Ctr1 was used to increase iron uptake (Froquet et al. 2001). Iron impairs the growth of biofilm in vivo (Zhang et al. 2008).

In C. albicans, iron-mediated metabolism was shown to be a major virulence factor (Et et al. 2010). Ctr1 was found to be essential for growth and virulence in C. albicans. In addition, Ctr1 was not only required for virulence but also for virulence in an in vivo animal model (Zhang et al. 2008). In addition, Ctr1 was found to be a member of the transporters Ctr1 and Ctr4 (Zhang et al. 2008). Deletion of Ctr1 severely affects the biofilm formation and biofilm formation of C. albicans. These data suggest that Ctr1 is involved in the iron-sensing and metabolic pathways in C. albicans.

In the present study, the iron-sensing and iron-transport systems in C. albicans were examined in a. reilianum, an ascomycete fungal pathogen. In addition, the transcriptional response of C. albicans to iron starvation was examined in vitro and in vivo. Finally, C. albicans mutants lacking Ctr1 displayed a delayed biofilm formation. In all studies, C. albicans exhibited a constitutive iron-responsive transcriptome and a constitutive iron-responsive protein expression profile. The transcriptome analysis revealed that C. albicans is a multicellular organism and differentiates it from other fungi. This transcriptomics study provides new insights into the iron regulatory pathways in C. albicans and identifies a key role in the virulence of this important pathogen.

Methods

The reaction was stopped by adding 1 M NaOH and the precipitate was collected after centrifugation at 5,000 g for 5 min at 4°. The pellet was washed with a 1 ml Tris-HCl (pH 8.0) solution and freeze-dried. The ferric

ferrous sulfate (FGS) fraction was precipitated with 1 M Tris-HCl (pH 8.0) and freeze-dried. The combined samples were dialyzed against distilled water for 1 h at 4°C to remove precipitate. The resulting material was dialysed against water for 1 h at 4°C, and the resulting mixtures were treated with 20 mM dithiothreitol (DTT) for the separation of the corresponding hydrocarbons. The ferric ferrous sulfate (FGS) fraction was precipitated with 1 M dithiothreitol (DTT) and freeze-dried. The mixture was treated with 20 mM DTT and dried by centrifugation at 4°C. The final product was dialysed against 1 M sodium cacodylate buffer (pH 8.0) and dried by centrifugation at 4°C. The resultant material was used for the determination of the ferric ferrous sulfate (FGS) by HPLC.

Isolation and characterization of Pce1 Pce1 was isolated from the cell culture of Aspergillus niger. Cells were cultured in YPD liquid medium with or without 2% agar. Cells were cultured at 25°C in a rotary shaker at 200 rpm for 24 h. The number of cells was counted with a hemocytometer. Then, the cells were collected by centrifugation at 2,000 g for 5 min at 4°C. The cell pellet was washed with distilled water and dried on ice. The cell pellet was frozen with liquid nitrogen and stored at -80°C until use.

Molecular typing of Pce1 gene The Pce1 gene was amplified from the pET-21a genomic DNA using the primer pair Pce1-F (5'-CCTCTCCGCGGCTCC-3') and Pce1-R (5'-CCGCTCTCTTCAGCCAT-3') (P.G. Hester et al., 1997) with the following changes: 0.

Results

To understand the molecular basis for Pce1-dependent transcriptional regulation in M. oryzae, we examined PCE1 gene expression in three independent yeast strains, namely the pce1Δ mutant, pce1Δ/pce1Δ, and the pce1Δ/pce1Δ/pce1Δ/pce1Δ complemented strain. Both strains exhibited similar expression profiles of PCE1 genes in two independent Pce1 deletion strains (Fig 6A), and Pce1 deletion complemented strains showed similar gene expression profiles (Fig 6B). These data suggest that Pce1 is an important regulator of the iron metabolism pathway and that Pce1 is not solely responsible for the regulation of the iron-sulfur symporter.

PCE1 is required for the growth of M. oryzae in the presence of high $FeCl_3$ concentrations To further examine the role of PCE1 in iron homeostasis, Pce1 and Pce1-overexpressing mutants were grown in the presence of iron concentrations ranging from 0.5 to 1 mM $FeCl_3$. As shown in Fig 7, the Pce1-overexpressing strain grew normally in the presence of $FeCl_3$, while the Pce1-overexpressing strain grew poorly in the presence of $FeCl_3$ (Fig 7A). PCE1 overexpression did not result in any significant growth defect in the presence of 0.5 mM $FeCl_3$, although growth of the complemented strain was similar to that of the PCE1-overexpressing strain. These data suggest that Pce1 plays a key role in iron homeostasis by controlling iron acquisition by iron uptake and detoxification.

Inhibition of Pce1 induces changes in iron uptake by altering the concentration of $FeCl_3$ Iron acquisition by Pce1 and Pce1-overexpressing mutants is influenced by $FeCl_3$ concentrations. To determine whether Pce1 can directly or indirectly inhibit $FeCl_3$ uptake, we tested PCE1 overexpression in the presence and concentration of $FeCl_3$.

Discussion

The expression of Pce1 in Aspergillus fumigatus was also examined in a separate set of nine fungal isolates (Table S1. All of these isolates are pathogenic and have been shown to produce the γ-ATPase Pce1 that is required for the biosynthesis of ferricrocin (21). Pce1 is also required for the biosynthesis of luteolin (18). The reason for this difference is not clear but may be due to differences in gene expression in these strains.

Overall, the results obtained using these nine isolates are consistent with those reported previously by Sakamoto et al. (17) and our own (20). These isolates are characterized by the same genetic properties as A. fumigatus (Fusarium spp. and Aspergillus spp.) and thus they are suitable for further analysis.

In order to examine the potential relationship between the Pce1 and Aspergillus genomes, we used the complete Pce1 genome of A. fumigatus, which contained the entire set of Pce1 genes (Table S2). The Aspergillus genome has been previously reported as being more compact than A. fumigatus, which is in fact true, as shown in Table S3, and is therefore in fact enriched in genes that encode for proteins involved

in many processes including cell differentiation, apoptosis, cell-cycle progression, cell wall synthesis, and stress responses (24). The Aspergillus genome also has been reported to have three genes involved in the biosynthesis of sterol-related compounds (Table S4). All three genes are involved in the biosynthesis of sterol-containing secondary metabolites (20). The biosynthesis of sterol-containing compounds is the basis for several fungal virulence genes, including sterol biosynthesis (20).

Comparison of the Pce1 and Aspergillus genomes revealed that the Aspergillus genome has been significantly expanded compared to the A. fumigatus genome (Table S5). This result suggests that Aspergillus is more resistant to the antifungal treatments and is thus more likely to be included in the pathogenicity analysis.