In vitro response of clinical isolates of Candida species to oxidative stress

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ABSTRACT: (In vitro response of clinical isolates of Candida species to oxidative stress). Pathogenic species of Candida differ in many aspects, including their clinical prevalence, virulence, and profile of antifungal resistance. One of the causes of these differences is possibly related to the differential capacity of these species to deal with oxidative stress. In this study, we compared clinical isolates of eight species of Candida with respect to their oxidative stress resistance in vitro, oxidative damage induction, and antioxidant enzymes. Intraspecific and interspecific variation was observed. In accord with data previously obtained from laboratory isolates, the results here indicate that C. albicans, C. glabrata and C. krusei have a more effective antioxidant system, and that C. dubliniensis, C. famata and C. guilliermondii are highly sensitive to oxidative stress. C. parapsilosis and C. tropicalis have intermediate resistance profiles. The stronger antioxidant system of some species may enable them to cause systemic infections or to resist antifungals.

Keywords: Candida species, oxidative stress, antioxidant defenses.

INTRODUCTION

The clinical spectrum of infections caused by species of Candida ranges from benign colonization of the skin and mucosal surfaces to mucocutaneous forms of candidiasis and systemic infections (Netea et al. 2008, Favalessa et al. 2010). Sobel (2010) pointed out that, for more than a decade, multiple epidemiological studies have indicated that Candida species are the fourth most common cause of nosocomial bloodstream infection worldwide; emphasis was given to the continued extremely high mortality associated with candidemia, which approaches 35% in the United States (Carlisle et al. 2009, Sobel 2010).

Some factors involved in the development of candidiasis are: mucosal and cutaneous barrier disruption, neutrophil dysfunction (quantitative and qualitative), metabolic disorders, and extremes of age (< 1 and > 70 yr) (Pfaffer & Diekema 2007). AIDS patients, organ transplant recipients, cancer patients receiving chemotherapy, recipients of artificial joints and prosthetic devices, and other immunocompromised individuals are particularly susceptible to candidiasis (Carlisle et al. 2009).

Although Candida albicans remains the most important human fungal pathogen because of its frequency of isolation (do Couto et al. 2011), and the amount of morbidity and mortality that it causes, the relative prevalence of species of Candida has changed (Sobel 2010). Horn et al. (2009) described a higher incidence of non-albicans species (54.4%) than C. albicans (45.6%) in 2019 candidemia cases. C. tropicalis and C. parapsilosis are highly important in the hospital environment (Storti et al. 2012). The decline in relative occurrence of C. albicans has largely been the result of an increased proportion of C. glabrata (Sobel 2010).

The initial host response to Candida is through recognition by innate immune cells (especially dendritic cells, macrophages, and neutrophils) and subsequent phagocytosis and elimination (Brown 2005). Professional phagocytes destroy pathogens in part through reactive oxygen species (ROS), including the superoxide anion (O₂⁻), hydrogen peroxide (H₂O₂) and the hydroxyl radical...
(OH), generated directly or indirectly by nicotinamide adenine dinucleotide phosphate-oxide (NADPH) oxidase (Flannagan et al. 2009). The importance of ROS in pathogen elimination is highlighted by individuals with mutations that cause partial or total inactivation of NADPH oxidase, and who as a consequence suffer from chronic granulomatous disease. This disease is characterized by severe, recurrent, life-threatening infections. In addition, incontrovertible evidence of the antimicrobial roles of O$_2^-$ and H$_2$O$_2$ has been established from the results of targeted deletion of the antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD) (Wysong et al. 1998, Marchenko et al. 2004).

Butler et al. (2009) showed recently that there are significant expansions of gene families related to virulence in pathogenic Candida species. The occurrence of genes involved in stress responses is also variable among species. Previous studies have also demonstrated differences among Candida species in their capacity to overcome ROS toxicity (Tosello et al. 2007, Cuéllar-Cruz et al. 2008). Our previous results also revealed some differences in responses to oxidative stress (Abegg et al. 2010). Here, we analyzed in vitro oxidative stress responses of clinical isolates of eight important pathogenic Candida species.

**MATERIALS AND METHODS**

**Yeast Strains, Media, and Culture Conditions**

Yeast isolates studied were: C. albicans 1 (isolate from a nosocomial patient), C. albicans 51 (orotracheal tube of an AIDS patient), C. dubliniensis 23 and C. dubliniensis 25 (both from the oropharynx of AIDS patients), C. famata 1 and C. famata 24 (both clinical isolates from nosocomial patients), C. glabrata 1, C. glabrata 75, and C. glabrata 118 (all obtained from catheter tips), C. guilliermondii 73 (clinical isolate from a nosocomial patient) and C. guilliermondii 6260 (American Type Culture Collection - ATCC isolate from a patient with broncho-mycosis), C. krusei 1 and C. krusei 2 (both isolated from skin lesions of diabetic patients), C. parapsilosis 81 and C. parapsilosis 115 (both isolated from patients with onychomycosis) and C. tropicalis 1 (isolated from an oral granuloma), and C. tropicalis 55 and C. tropicalis 56 (both clinical isolates from nosocomial patients). The maintenance of the yeasts on solid yeast extract-peptone-dextrose (YPD) medium (4-8 °C) and species identification of the isolates by means of morphological and biochemical tests were based on Kurtzman et al. (2011).

Viable cells obtained from yeast cultivated on solid YPD medium were grown in liquid YPD medium on an orbital shaker (30 °C, 100 rpm) until exponential growth was reached (OD$_{600nm}$ = 1.5-1.6). Cells were then washed and diluted to OD$_{600nm}$ = 0.15 in fresh liquid YPD for use. To obtain cell-free extracts, cells were lysed by disruption with glass beads and then centrifuged (10 min, 8,000 g) to remove debris.

**Oxidant Sensitivity Assays**

**Disk diffusion tests**

For disk diffusion tests, sterile 6-mm diameter filter paper disks were used. Each disk was impregnated with 5 µl of 7.5 M H$_2$O$_2$ (freshly opened bottles were always used), 0.5 M paraquat (1,1V-dimethyl-4,4V-bipyridinium dichloride hydrate, 95% purity) or 0.3 M menadione (2-methyl-1,4-naphthoquinone, vitamin K3, 95% purity). Diluted yeast cells (OD$_{600nm}$ = 0.15) were plated onto YPD solid agar by means of sterile cotton swabs and incubated at 30 °C for up to 72 h with the disks. Halos of growth inhibition were measured with a micrometer (Lamarre et al. 2001).

**Spot tests**

For spot tests, initial cell suspensions were diluted up to 10$^{-4}$ in sterile water and spotted (5 µl) onto YPD agar plates containing 12 mM H$_2$O$_2$. After 72 h at 30 °C the growth of each isolate was examined and the plates were photographed (Abegg et al. 2010).

**MDA Determination**

Malondialdehyde (MDA) was measured by HPLC using a method described by Karatepe (2004). The chromatograms were monitored at 250 nm.

**Protein Carbonyl Groups**

The protein carbonyl groups were quantified by a method employing 2,4-dinitrophenylhydrazine (DNPH) (Levine et al. 1990) as described elsewhere (Abegg et al. 2010).

**Enzymatic Assays**

For enzymatic assays, cell suspensions were treated with 0.5 mM H$_2$O$_2$ (final concentration) or left untreated, and incubated for 1 h, 30 °C, 100 rpm on a rotary platform. Cells were then washed, lysed and centrifuged. Clarified supernatants were used in enzymatic assays.

Catalase (EC 1.11.1.6) activity was determined by monitoring the removal of H$_2$O$_2$, as proposed by Aebi (1984).

Glutathione peroxidase (GPx - EC 1.11.1.9) activity was assayed using 10 µl samples of the supernatants with the RANSEL commercial kit (Randox® Laboratories, Crumlin, UK). Superoxide dismutase (EC 1.15.1.1) activity was measured in 10 µl samples of cell supernatant using the RANSOD commercial kit (RANSOD SD 125, Randox® Laboratories, Crumlin, UK) according to the manufacturer’s protocol.

**Total Protein Content**

To normalize the results, the total protein content of the cell extracts was quantified using the Bradford method (1976).

**Data Analysis**

Results are expressed as means ± S.D. Statistical analysis was performed using PASW 18.0 software (SPSS, Chicago, IL, USA). To compare variables between

groups, a one-way ANOVA was performed, followed by the post-hoc test of Tukey. Untreated samples were compared with treated samples by Student’s t-test. Correlations were determined by Spearman rank correlation coefficient (rho). Values of $P \leq 0.05$ were considered statistically significant. In the figures and Table 1, statistics have been omitted to facilitate interpretation.

RESULTS

Disk diffusion and spot tests were used to compare the relative sensitivity of the isolates to oxidants (Fig. 1 and Fig. 2). The C. albicans and C. krusei 2 isolates were significantly more resistant to menadione than the C. dubliniensis and C. guilliermondii isolates. The C. glabrata isolates were significantly more resistant to H$_2$O$_2$ than were C. dubliniensis 23 and C. guilliermondii 6260. The C. albicans isolates were significantly more resistant to parquat than were C. famata 1 and C. guilliermondii 6260 (Fig. 1).

Spots obtained without oxidative treatment and with 12 mM H$_2$O$_2$ YPD plates are shown in Figure 2a and 2b, respectively. The C. dubliniensis, C. famata and C. guilliermondii isolates were the most sensitive to H$_2$O$_2$. The other species were more resistant and grew better under H$_2$O$_2$ stress (Fig. 2b).

MDA levels were determined as a means to evaluate the lipid peroxidation index, and the degree of protein damage was assessed by measurement of protein carbonylation. The C. parapsilosis and C. tropicalis isolates showed the highest MDA levels. MDA levels in the C. parapsilosis and C. tropicalis isolates were significantly higher than in the C. krusei isolates. The clinical C. guilliermondii isolates showed carbonyl levels that were significantly higher than in the C. albicans isolates.

CAT, GPx, and SOD activities were determined with and without oxidative treatment (0.5 mM H$_2$O$_2$) (Table 1). Oxidative treatment increased CAT activity in all species tested. C. albicans, C. glabrata and C. krusei demonstrated the highest CAT activity. We found a reduction in GPx activity in most of the isolates under the conditions employed. SOD activity either was increased or was not significantly altered in most of the isolates (Student t-test; $P > 0.05$) (Table 1). The C. parapsilosis isolates had the highest SOD activity, followed by the C. glabrata isolates.

Table 1. Antioxidant enzyme activities in Candida extracts.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Treatment</th>
<th>GPx (U/mg ± SD)</th>
<th>SOD (U/mg ± SD)</th>
<th>Catalase (U/g ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans 1</td>
<td>Without H$_2$O$_2$</td>
<td>358.6±12.8</td>
<td>53.6±0.4</td>
<td>532.5±43.2</td>
</tr>
<tr>
<td></td>
<td>0.5 mM H$_2$O$_2$</td>
<td>99.9±5.9</td>
<td>6.7±0.2</td>
<td>7253.5±539.9</td>
</tr>
<tr>
<td>C. albicans 51</td>
<td>Without H$_2$O$_2$</td>
<td>351.2±9.7</td>
<td>7.7±0.2</td>
<td>294.2±43.5</td>
</tr>
<tr>
<td></td>
<td>0.5 mM H$_2$O$_2$</td>
<td>74.9±11.8</td>
<td>7.1±0.3</td>
<td>3681.3±622.5</td>
</tr>
<tr>
<td>C. dubliniensis 23</td>
<td>Without H$_2$O$_2$</td>
<td>80.9±12.9</td>
<td>11.4±1.0</td>
<td>282.4±15.9</td>
</tr>
<tr>
<td></td>
<td>0.5 mM H$_2$O$_2$</td>
<td>99.8±7.4</td>
<td>7.3±2.7</td>
<td>1511.5±356.3</td>
</tr>
<tr>
<td>C. dubliniensis 25</td>
<td>Without H$_2$O$_2$</td>
<td>204.4±22.3</td>
<td>7.2±0.3</td>
<td>154.2±19.8</td>
</tr>
<tr>
<td></td>
<td>0.5 mM H$_2$O$_2$</td>
<td>121.8±25.3</td>
<td>5.7±0.1</td>
<td>158.9±43.4</td>
</tr>
<tr>
<td>C. famata 1</td>
<td>Without H$_2$O$_2$</td>
<td>177.5±32.7</td>
<td>6.1±0.2</td>
<td>326.7±31.7</td>
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<tr>
<td></td>
<td>0.5 mM H$_2$O$_2$</td>
<td>58.5±17.8</td>
<td>6.0±0.2</td>
<td>4867.8±656.3</td>
</tr>
<tr>
<td>C. famata 24</td>
<td>Without H$_2$O$_2$</td>
<td>249.3±45.9</td>
<td>4.6±0.5</td>
<td>308.8±102.9</td>
</tr>
<tr>
<td></td>
<td>0.5 mM H$_2$O$_2$</td>
<td>65.2±11.9</td>
<td>4.1±0.1</td>
<td>2228.6±335.6</td>
</tr>
<tr>
<td>C. glabrata 1</td>
<td>Without H$_2$O$_2$</td>
<td>154.4±34.5</td>
<td>3.4±0.2</td>
<td>3554.7±695.9</td>
</tr>
<tr>
<td></td>
<td>0.5 mM H$_2$O$_2$</td>
<td>126.7±12.8</td>
<td>3.5±0.2</td>
<td>8286.5±260.9</td>
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<tr>
<td>C. glabrata 75</td>
<td>Without H$_2$O$_2$</td>
<td>219.7±88.8</td>
<td>2.8±0.3</td>
<td>749.5±177.9</td>
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<tr>
<td></td>
<td>0.5 mM H$_2$O$_2$</td>
<td>198.9±18.7</td>
<td>7.7±0.1</td>
<td>5868.8±1082.6</td>
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<tr>
<td>C. glabrata 118</td>
<td>Without H$_2$O$_2$</td>
<td>332.8±104.5</td>
<td>8.9±0.3</td>
<td>520.2±104.0</td>
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<td></td>
<td>0.5 mM H$_2$O$_2$</td>
<td>78.0±17.8</td>
<td>12.3±0.4</td>
<td>2130.0±134.6</td>
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<tr>
<td>C. guilliermondii 73</td>
<td>Without H$_2$O$_2$</td>
<td>396.0±88.4</td>
<td>5.6±0.2</td>
<td>123.9±56.7</td>
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<tr>
<td></td>
<td>0.5 mM H$_2$O$_2$</td>
<td>276.5±35.6</td>
<td>4.7±0.2</td>
<td>1409.6±282.4</td>
</tr>
<tr>
<td>C. guilliermondii 6260</td>
<td>Without H$_2$O$_2$</td>
<td>397.9±28.9</td>
<td>10.0±0.2</td>
<td>147.8±29.6</td>
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<tr>
<td></td>
<td>0.5 mM H$_2$O$_2$</td>
<td>239.8±65.7</td>
<td>6.8±1.9</td>
<td>2921.6±177.0</td>
</tr>
<tr>
<td>C. krusei 1</td>
<td>Without H$_2$O$_2$</td>
<td>249.6±13.7</td>
<td>4.1±0.5</td>
<td>159.4±21.4</td>
</tr>
<tr>
<td></td>
<td>0.5 mM H$_2$O$_2$</td>
<td>128.7±11.9</td>
<td>6.6±1.0</td>
<td>5702.0±691.2</td>
</tr>
<tr>
<td>C. krusei 2</td>
<td>Without H$_2$O$_2$</td>
<td>480.5±22.6</td>
<td>9.9±1.1</td>
<td>203.6±26.2</td>
</tr>
<tr>
<td></td>
<td>0.5 mM H$_2$O$_2$</td>
<td>376.8±67.8</td>
<td>6.8±0.8</td>
<td>4357.5±568.8</td>
</tr>
<tr>
<td>C. parapsilosis 81</td>
<td>Without H$_2$O$_2$</td>
<td>413.9±6.8</td>
<td>11.3±2.6</td>
<td>236.4±81.9</td>
</tr>
<tr>
<td></td>
<td>0.5 mM H$_2$O$_2$</td>
<td>120.8±39.9</td>
<td>14.8±2.2</td>
<td>3032.8±772.5</td>
</tr>
<tr>
<td>C. parapsilosis 115</td>
<td>Without H$_2$O$_2$</td>
<td>275.3±77.9</td>
<td>7.1±0.1</td>
<td>732.8±60.2</td>
</tr>
<tr>
<td></td>
<td>0.5 mM H$_2$O$_2$</td>
<td>121.3±15.3</td>
<td>13.5±1.0</td>
<td>2347.9±133.8</td>
</tr>
<tr>
<td>C. tropicalis 1</td>
<td>Without H$_2$O$_2$</td>
<td>356.9±38.8</td>
<td>7.0±0.4</td>
<td>257.1±17.3</td>
</tr>
<tr>
<td></td>
<td>0.5 mM H$_2$O$_2$</td>
<td>54.1±9.9</td>
<td>6.9±0.1</td>
<td>1433.9±98.5</td>
</tr>
<tr>
<td>C. tropicalis 55</td>
<td>Without H$_2$O$_2$</td>
<td>422.9±61.9</td>
<td>12.4±0.9</td>
<td>65.2±18.4</td>
</tr>
<tr>
<td></td>
<td>0.5 mM H$_2$O$_2$</td>
<td>114.3±6.9</td>
<td>15.1±1.7</td>
<td>1266±83.2</td>
</tr>
<tr>
<td>C. tropicalis 56</td>
<td>Without H$_2$O$_2$</td>
<td>288.2±31.3</td>
<td>4.0±0.4</td>
<td>296.5±52.3</td>
</tr>
<tr>
<td></td>
<td>0.5 mM H$_2$O$_2$</td>
<td>140.4±7.6</td>
<td>3.3±0.5</td>
<td>1891.0±78.8</td>
</tr>
</tbody>
</table>

Cultures of Candida species were grown aerobically at 30 °C in YPD medium at 100 rpm until early exponential phase. Cultures were then either exposed to H$_2$O$_2$ or left untreated, and then frozen at -80 °C. Cells were harvested and extracts were prepared and assayed as described in Materials and Methods. Values reported are the means of three determinations.

DISCUSSION

In this study, we tested the in vitro oxidative stress response of clinical isolates of Candida species. The disk diffusion test is widely employed to evaluate the sensitivity of microorganisms to oxidative agents (Lamarre et al. 2001, Sampaio et al. 2009). In agreement with the present results, Sampaio et al. (2009) also found significant differences in the susceptibility patterns of C. albicans to various stress agents. These authors found inhibition halos ranging from 30-35 mm using 10 µl of 8.8 M H₂O₂, compared to the 20-23 mm halos with 5 µl of 8.8 M H₂O₂ observed in this study. They also found halos ranging from 20-30 mm using 10 µl of 0.5 M menadione, compared to the 20 mm halos with 5 µl of 0.3 M menadione, observed in this study (Fig. 1).

Our results for growth of C. albicans under 12 mM H₂O₂ stress were equivalent to those under 8 mM H₂O₂ stress obtained by Walia and Calderone (2008). Taken together, the results of the sensitivity assays generally agree with our previous observation that C. albicans, C. glabrata and C. krusei are particularly resistant to oxidative stress, that C. parapsilosis and C. tropicalis possess an intermediate degree of resistance, and that C. dubliniensis, C. famata and C. guilliermondii exhibit more sensitivity to in vitro oxidative stress (Abegg et al. 2010). Costa-de-Oliveira et al. (2012) also observed that C. krusei isolates showed a high degree of resistance to oxidative stress, and these investigators found the presence of an alternative oxidase (AOX) in C. krusei, which may be related to this resistance.

MDA and lipid peroxidation assays were conducted under mild oxidative stress (0.5 mM H₂O₂) according to the method of Srinivasa et al. (2012) (Fig. 3a and 3b). MDA levels did not correlate with carbonyl levels. However, carbonyl levels correlated with the sensitivity of the isolates in disk diffusion tests (rho = 0.424, 0.300, and 0.411 for menadione, H₂O₂, and paraquat, respectively). The production of MDA depends on the availability of substrates, which are usually membrane polyunsaturated fatty acids (PUFA) (Antunes et al. 1996). C. albicans possesses membrane PUFA (Murayama et al. 2006), but little is known about the PUFA content in other pathogenic Candida species. The increase in CAT activity following oxidative treatment was higher than our previous observation (Abegg et al. 2010), and may contribute to the higher resistance to oxidative stress observed in species such as C. albicans, C. glabrata and C. krusei, which demonstrated higher activity (Table 1). Similar increases in CAT activity with oxidative stress have been observed previously (Jamieson et al. 1996, González-Párraga et al. 2003, Tosello et al. 2007).

Kusch et al. (2007) observed an up-regulation in CAT and GPx proteins after treatment with 1 mM H₂O₂. However, Yang et al. (2009), investigating the influence of culture conditions on GPx production in C. albicans, observed a slight reduction in GPx activity using 0.1 mM H₂O₂ and a slight elevation using 1 mM H₂O₂. This seems to demonstrate that, in addition to the culture conditions, the different concentrations of the oxidant employed strongly influenced the antioxidant activity of GPx (Jamieson et al. 1996, González-Párraga et al. 2003).

The possible inhibition of SOD activity under mild oxidative stress is in agreement with the results of Gunasekaran et al. (1998). These authors found a slight reduction in SOD activity using 1 mM H₂O₂ (from 15.05 to 12.46 U/mg protein), and an 83.6% reduction using 50 mM H₂O₂. Pedreño et al. (2006) observed a reduction in SOD activity in yeast treated with 5 mM H₂O₂. However, Tosello et al. (2007) described a twofold increase in SOD activity in C. albicans and C. dubliniensis isolates...
**Figure 2.** Resistance to H$_2$O$_2$ of Candida species clinical isolates during logarithmic growth. Mid-log phase cultures of Candida species were diluted to OD$_{600}$ nm = 0.15. Dilutions up to 10$^{-4}$ in sterile water were spotted (5 µL) onto YPD agar plates without oxidant (A) or onto YPD agar plates containing 12 mM H$_2$O$_2$ (B). Plates were incubated at 30 °C for up to 72 h and photographed.
treated with 0.4 mM H$_2$O$_2$. Fekete et al. (2007) observed SOD activities ranging from 3.7 to 11 U/mg protein in untreated and treated (1 to 6 mM of tert-butyl hydroperoxide - t-BOOH) cells of *C. albicans*. Gyetvai et al. (2007) observed similar SOD activities in untreated cells of *C. albicans*. In general, the SOD activities reported here agree with those found in other studies.

Brown et al. (2009) noted that *in vitro* experimental data on nitrosative and oxidative stress responses must be interpreted with caution. Stress-resistance mechanisms other than scavenging of ROS by redox-reactive molecules may be used by *Candida* species. This was proposed by Wellington et al. (2009), who observed that *C. albicans* and *C. krusei* were highly effective in suppressing ROS production by phagocytes through a mechanism that was thought to be independent of ROS scavenging. Also, yeast cells are probably exposed simultaneously to combinations of different stresses in their natural environments rather than to individual stresses in isolation. Kaloriti et al. (2012) compared combinatorial stress responses in *C. glabrata* and *C. albicans*, showing that combined stresses, particularly combinations of osmotic plus oxidative and oxidative plus nitrosative stress, are especially potent against these two species.

Brown et al. (2014) recently reviewed the multiple and complex mechanisms that *C. albicans* cells employ in stress adaptation. The antioxidant enzymatic activities determined in the present study may be an important aspect of the stress response.

Suppression of ROS production was less effective in *C. glabrata* and *C. tropicalis* than in other *Candida* species (MacCallum 2008, Wellington et al. 2009). This suggests...
that certain Candida species use additional mechanisms to subvert the toxic effects of phagocytes. Recently, Bruce et al. (2011) identified and characterized a novel response regulator in C. albicans, termed Crl1 (Candida Response Regulator 1), that is not conserved in S. cerevisiae or S. pombe. They demonstrated that Crl1 is specifically involved in the response of C. albicans to hydrogen peroxide stress, but not to other oxidizing agents or a range of other stress conditions.

Haynes (2001) posed some questions yet to be answered on the subject: “Why is C. albicans a more prevalent pathogen than other Candida species? Is it more widespread and the prevalence is just a reflection of this, or does C. albicans have a different repertoire of virulence determinants compared to other Candida species that allow it to be a better pathogen?” Our view is that species such as C. albicans and C. glabrata have a larger repertoire of virulence determinants despite the fact that, for example, Candida tropicalis and Candida parapsilosis are good producers of acid protease and phospholipase B, and species with a stronger antioxidant capacity are better prepared to cause disseminated infections. C. albicans, C. glabrata and C. krusei probably developed multiple evasion mechanisms in order to survive in the host.

Considering that, in vivo, C. albicans (Brown et al. 2014) and other pathogenic Candida species occupy complex and dynamic host niches characterized by alternative carbon sources and simultaneous exposure to combinations of stresses, different aspects of in vivo pathogenicity such as adhesion, biofilm formation and cell morphology are likely to be closely related to the pathogenicity such as adhesion, biofilm formation and species with a stronger antioxidant capacity are better prepared to cause disseminated infections. C. albicans, C. glabrata and C. krusei probably developed multiple evasion mechanisms in order to survive in the host.

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