Major Article

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Glutathione levels in and total antioxidant capacity of *Candida* sp. cells exposed to oxidative stress caused by hydrogen peroxide

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ABSTRACT

Introduction: The capacity to overcome the oxidative stress imposed by phagocytes seems to be critical for *Candida* species to cause invasive candidiasis. **Methods:** To better characterize the oxidative stress response (OSR) of 8 clinically relevant *Candida* sp., glutathione, a vital component of the intracellular redox balance, was measured using the 5,5'-dithiobis-(2-nitrobenzoic acid (DTNB)-glutathione disulfide (GSSG) reductase reconversion method; the total antioxidant capacity (TAC) was measured using a modified method based on the decolorization of the 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic) acid radical cation (ABTS*+). Both methods were used with cellular *Candida* sp. extracts treated or not with hydrogen peroxide (0.5 mM). **Results:** Oxidative stress induced by hydrogen peroxide clearly reduced intracellular glutathione levels. This depletion was stronger in *Candida albicans* and the levels of glutathione in untreated cells were also higher in this species. The TAC demonstrated intra-specific variation. **Conclusions:** Glutathione levels did not correlate with the measured TAC values, despite this being the most important non-enzymatic intracellular antioxidant molecule. The results indicate that the isolated measurement of TAC does not give a clear picture of the ability of a given *Candida* sp. to respond to oxidative stress.

Keywords: *Candida* sp. Glutathione. Total antioxidant capacity.

INTRODUCTION

Candida albicans, *C. glabrata*, *C. tropicalis,* and *C. parapsilosis* account for approximately 95% of identifiable *Candida* infections. Other species, including *C. krusei*, *C. lusitaniae,* and *C. guilliermondii*, account for less than 5% of cases of invasive candidiasis. The most common causative agent is still *C. albicans*, but its incidence is declining and the frequencies of other species are increasing. Recently, Furlaneto et al.[1] noted that non-*albicans Candida* was the predominant species in different clinical specimens, with the exception of urine samples, in a Brazilian tertiary-care hospital. Invasive candidiasis has a mortality rate that approaches 40%[2,3]. Although most people are colonized by *Candida* sp., the majority never develop invasive candidiasis. Alterations in host immunity, physiological features, or normal microflora, rather than the acquisition of novel or hypervirulent factors by *Candida,* are suggested to degenerate the commensal-host interaction and lead to an opportunistic infection[4].

During the course of a systemic infection, *Candida* cells are engulfed by host phagocytes, where they are exposed to reactive oxygen species (ROS)[5]. ROS contribute to the killing of *C. albicans* in both cultured cells and entire organisms[6-9]. Upon incubation with macrophages, *C. albicans* deoxyribonucleic acid (DNA) repair genes are transcriptionally induced, suggesting that DNA damage indeed occurs in the phagosome and that genotoxic hypersensitivity stress would be disadvantageous to the pathogen[10]. Recently, it was demonstrated that a large proportion of *C. albicans* cell surface antigens related to acute candidemia are involved in oxidative stress[4]. In *C. albicans*, hyphal cells are more resistant to oxidative stress[10], and hyphal formation is higher in isolates resistant to azole drugs[11]. Taking into account these data, overcoming the oxidative phagocytic challenge seems to be critical for the establishment of candidemia.

Candida species have evolved an antioxidant defensive response in order to withstand ROS attack, which encompasses, among other components, glutathione (GSH, L-γ-glutamyl-L-cysteinyl-glycine) and GSH-related activities (i.e., glutathione reductase, glutathione peroxidase, and glucose-6P-dehydrogenase)[12]. GSH is the most abundant non-protein thiol in eukaryotic cells and its very low redox potential (E'_o = -240mV) provides the cell with redox buffer properties. In budding yeasts, GSH and its oxidized disulfide form (GSSG) are involved in essential physiological functions, such as DNA and protein synthesis, transport, and cellular detoxification[13]. Yeast isolates lacking glutathione or that have altered glutathione redox states are sensitive to peroxide-induced oxidative stress, superoxide anions, and lipid peroxidation products[13-16].

Numerous assays have been described to measure antioxidant status, but it seems that no ideal method is available[17]. Different antioxidants can be measured separately, but the measurements are time-consuming, labor-intensive, costly, and often require complicated techniques[18,19]. Hence, the concept of a single test that might reflect the total antioxidant capacity (TAC) of biological fluids has elicited interest. The most widely used colorimetric methods to measure TAC are 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic) acid radical cation (ABTS*+)-based methods. Reduced ABTS, a colorless molecule, is oxidized to ABTS*+, which is characteristically blue-green. When this radical is mixed with any oxidizable substance, it is reduced to its colorless form[18].

Different *Candida* sp. exhibit unequal oxidative stress resistances *in vitro*[20-22], and different *in vitro* virulence potentials[23], and we proposed that this may contribute to the capacity of each species to cause candidemia[22]. Taking into account these differences, total glutathione levels and the cellular TAC were assessed in 8 *Candida* species.

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METHODS

Yeast isolates and cultivation

The following yeast isolates were used: *C. albicans* type strain ATCC 18804, *C. dubliniensis* type strain from the Centraalbureau voor Schimmelcultures (CBS) 7987, *C. famata* ATCC 62894, *C. glabrata* type strain ATCC 2001, *C. guilliermondii* type strain ATCC 46036, *C. guilliermondii* ATCC 6260, *C. krusei* type strain ATCC 6258, *C. parapsilosis* type strain ATCC 22019, and *C. tropicalis* ATCC 13803. The clinical isolates used were as follows: *C. albicans* 1 (isolate from a patient with nosocomial infection); *C. albicans* 51 (isolate from the orotracheal tube of an acquired immunodeficiency syndrome (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 patients with nosocomial infection); *C. glabrata* 1, *C. glabrata* 75, and *C. glabrata* 118 (all obtained from catheter tips); *C. guilliermondii* 73 (clinical isolate from a patient with nosocomial infection); *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); *C. tropicalis* 1 (isolated from an oral granuloma); and *C. tropicalis* 55 and *C. tropicalis* 56 (both clinical isolates from a patient with nosocomial infection).

The isolates were identified and maintained as previously described[22]. Viable cells were obtained by cultivation on solid yeast extract-peptone-dextrose (YPD) medium (1% yeast extract, 2% peptone, 2% glucose, 2% agar), and isolates were then grown in liquid YPD medium in an orbital shaker at 30°C/100 rpm to late exponential growth (OD_{600nm} = 1.5–1.6). Cells were washed twice with sterile distilled water and diluted to OD_{600nm} = 0.15 in fresh liquid YPD for use. Cells were grown at 30°C rather than at 37°C because *C. dubliniensis* and *C. famata* grow better at 30°C.

Cell-free extracts

Cell suspensions (1.5mL) were centrifuged for 5 min at 8,000*g* and lysed by adding 0.5mL of lysis buffer (50mM Tris-Cl, 150mM NaCl, 50mM ethylenediamine tetraacetic acid [EDTA], pH 7.2), 50mM phenyl methyl sulfonyl fluoride (PMSF; Sigma, St. Louis, MO) and approximately 0.5 g of glass beads (diameter, 425-600µm; Sigma). Lysis was performed by vortexing for 3 mixing cycles of 3 min with 1-min intervals for cooling on ice. Breakage was checked microscopically. The samples were then centrifuged for 10 min at 8,000*g* to remove cellular debris and beads.

Total glutathione assay

Total intracellular glutathione was determined by the 5,5'-dithiobis-(2-nitrobenzoic acid (DTNB)-glutathione disulfide GSSG reductase recycling method[24,25]. Cell suspensions were left untreated or were treated with 0.5mM H_2O_2, incubated for 1h with agitation at 100rpm/30°C, washed with sterile distilled water, and then resuspended to the same volume in 100mM potassium phosphate buffer (pH 7.0), lysed, and centrifuged. Then, 25µL aliquots of the supernatants were vortexed thoroughly with an equal volume of 2M $HClO_4$ and 4mM EDTA. After 15 min incubation at 0°C, the suspensions were centrifuged for 5 min at 8,000*g* and 45µL of the supernatant was pH-neutralized by adding 3µL of 2M KOH at 0°C. This was centrifuged for 1 min at 8,000*g* and 35µL of the supernatant was added to a mixture containing 174µL of 100mM phosphate buffer (pH 7.0), 17µL of 4mM NADPH, and 7µL of glutathione reductase solution (6U/mL). This was mixed and incubated for 5 min at 37°C. Then, 18µL of DTNB reagent (0.040g of DTNB [Sigma] dissolved in 10ml of 50mM potassium phosphate buffer, pH 7.0) was added, and the absorbance was read at 412nm after a 2-min incubation at 37°C.

Total antioxidant capacity

A modified method based on ABTS*+ decolorization described by Erel[18] was employed. Cell suspensions were treated with 0.5mM H_2O_2, washed, lysed, and centrifuged, as previously described[22], and 5µL of each supernatant was mixed with 200µL of 0.4 mol/L acetate buffer, pH 5.8. Then, 20µL of ABTS*+ in 30mM acetate buffer, pH 3.6, was added, mixed, and the absorbance measured after 5 min. The absorbance of a solution without ABTS*+ was also measured as the blank. The vitamin E water-soluble analogue 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was used as the standard, and data were expressed in terms of mmol Trolox equivalent per milligram of protein.

Total protein content

The total protein content in lysed cells was determined by the Bradford assay (Bio-Rad, Hercules, CA).

Statistics

Statistical analyses were performed using the PASW software, v. 18.0 (SPSS, Chicago, IL). One-way ANOVA was performed, followed by the Tukey *post hoc* test to compare differences among groups. The Student's *t*-test was used to compare treated and untreated samples. Correlations were determined based on Spearman rank correlation coefficient (rho). Some statistical data have been omitted from the figures to facilitate visualization.

RESULTS

In the present work, the levels of total intracellular GSH following mild oxidative stress in *Candida* sp. were determined. GSH levels ranged from 80 to 290nmol/mg of protein in untreated samples and from 21 to 83nmol/mg of protein in treated samples **(Figure 1)**. With exception of *C. tropicalis*, all species tested exhibited a significant reduction in total GSH levels following exposure to mild oxidative stress (0.5mM H_2O_2). *C. albicans* presented the most dramatic reduction. In untreated samples, *C. albicans* presented the highest GSH levels and these levels were significantly higher than those seen in *C. dubliniensis*, *C. guilliermondii*, *C. krusei*, *C. parapsilosis,* and *C. tropicalis* ($p < 0.05$) **(Figure 1)**.

The TAC results were quite varied in each species **(Figure 2)**. One *C. albicans* isolate (51), 2 *C. guilliermondii* isolates (6260 and 73), and 1 *C. krusei* isolate (6258) presented the highest TAC levels. With exception to *C. guilliermondii* isolate 73 in comparison with *C. tropicalis* isolate 55, the isolates cited above exhibited significant differences in TAC levels compared to all other isolates tested ($p < 0.05$).

Concerning Spearman rank correlation coefficient, TAC results did not correlate (rho = 0.051) with sensitivity of *Candida* sp. isolates to oxidative stress. TAC results also did not correlate with total intracellular GSH levels in untreated (rho = 0.042) and treated (rho = 0.058) samples.

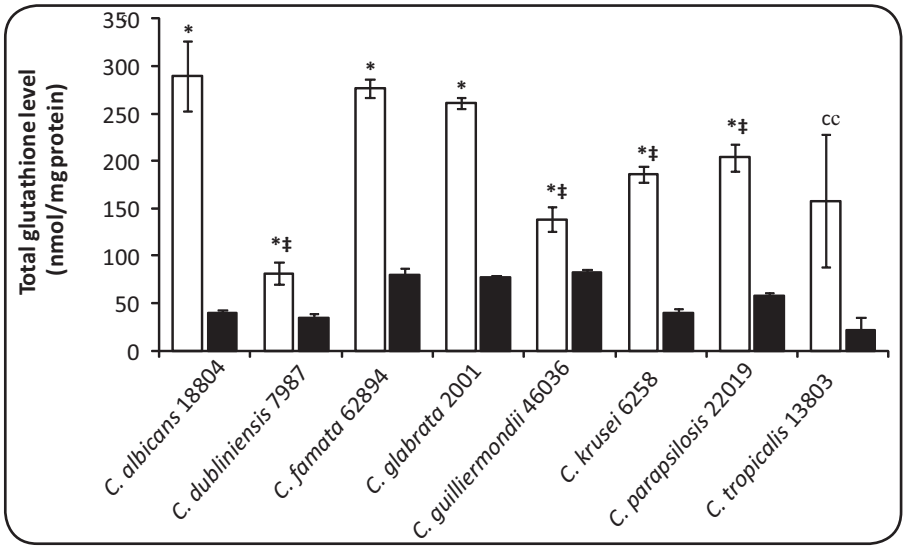

FIGURE 1 - Effect of 0.5 mM H_2O_2 on the total intracellular glutathione concentration (nmol (GSH + 2GSSG) mg^{-1} protein) in *Candida* sp. Cells were treated (black bars) or not (white bars), as described in Methods. Asterisks (*) indicate significant differences ($p < 0.01$) between untreated and treated samples. The symbol (‡) indicates significant differences between untreated *Candida albicans* cells and the isolates labeled. The data are the mean ± SD values of 3 independent experiments. **C:** *Candida.*

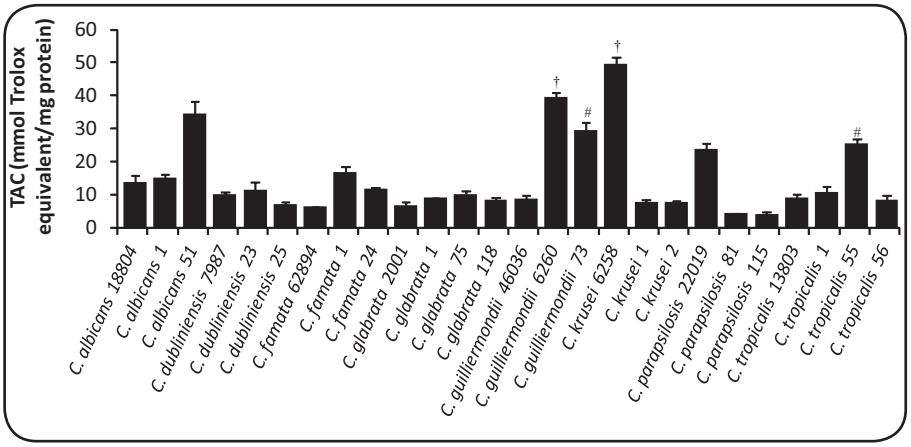

FIGURE 2 - Total antioxidant capacity in *Candida* sp. cells after 0.5mM H_2O_2 treatment. Cells were treated as described in Materials and Methods. Each column corresponds to the *Candida* isolate identified below. The symbol (†) indicates significant differences ($p < 0.05$) between the labeled isolate and all the other isolates tested. The symbol (#) indicates that the isolate *Candida guilliermondii* was not significantly different from the isolate *Candida tropicalis* 55 ($p > 0.05$), but was significantly different from all other isolates tested. The data represent the mean ± SD values of 3 independent experiments. ***C:** Candida;* **TAC:** total antioxidant capacity.

DISCUSSION

The virulence of *Candida albicans* seems to be multifactorial[26], but the ability of this fungus to mount stress responses is an important aspect, as this promotes survival in the host during systemic infections[27]. In a previous study by our group[22], we analyzed the oxidative effects (degree of resistance and induction of oxidative damage) and antioxidative effects (capacity to adapt and induction of antioxidative enzymes). Here, we continued the characterization of the oxidative stress response (OSR) of 8 clinically relevant *Candida* sp.

Hydrogen peroxide was used to generate oxidative stress. H_2O_2 itself is not very reactive, but can be further reduced to extremely damaging hydroxyl radicals. Therefore, all aerobic cells are equipped with H_2O_2-removing enzymes. Furthermore, evidence suggests that H_2O_2 is produced transiently in response to the activation of many cell surface receptors and serves as an intracellular messenger. The timely elimination of intracellular messengers after the completion of their mission is critical for receptor signaling. This would seem especially true for H_2O_2[28]. According to Ng et al.[29] the network of enzymes that detoxify H_2O_2 in biological systems has at least 3 nodes: catalase (which is the longest known enzyme for the removal of H_2O_2 and requires no cofactors), 6 members of the peroxiredoxin family of enzymes, and the glutathione peroxidases that rely on GSH as the electron donor and specific cofactor.

The GSH levels (90-152) observed by Fekete et al.[30] in untreated *C. albicans* isolates were similar to the levels found in this study **(Figure 1)**.

Consistent with this, Lemar et al.[31] showed that a 30-min *C. albicans* exposure to 0.5 mM diallyl disulfide (a garlic oxidative constituent) decreased intracellular GSH and elevated ROS intracellular levels. It was also demonstrated that H_2O_2 exposure causes a reduction in intracellular GSH levels, particularly for *Saccharomyces cerevisiae*, as well as a shift in the GSH redox balance towards the oxidized form, GSSG, as reviewed in Penninckx[13]. Thomas et al.[32] reported a dramatic decline in the level of intracellular GSH, concomitant with the yeast-to-mycelial conversion, in *C. albicans*. Consistent with this, Michán and Pueyo[33] reported that the GSH levels in *C. albicans* hyphae were approximately 50% of those in yeasts. Considering that oxidative stress diminishes GSH levels[33,34], our treatment with H_2O_2 was expected to reduce GSH content. Pacheco et al.[25] demonstrated that cadmium treatment increased ROS production, depleted intracellular GSH concentrations, and increased external GSH concentrations. Furthermore, González-Párraga et al.[12] used the oxidant 1-chloro-2,4-dinitrobenzene to reduce intracellular GSH levels in *Candida*. Madeo et al.[35] also demonstrated that treatment with 3 mM H_2O_2 induced intracellular GSH depletion and apoptosis in *S. cerevisiae*.

In contrast, Fekete et al.[36] found GSH levels of ~160nmol/mg of protein in untreated isolates of *C. albicans* and ~260nmol/mg of protein after treatment with 1mM tert-butil-hydroperoxide, an oxidant. Lee et al.[37] found that a 6-h treatment with 0.1mM H_2O_2 provoked a 3.14-fold elevation in GSH levels in *Schizosaccharomyces pombe*. Manfredini et al.[38] reported an increase in GSH levels upon treatment with 0.5mM H_2O_2 in wild-type *S. cerevisiae* cells and a significant reduction in those levels with 5mM H_2O_2. These differences regarding our results may be related to the duration of treatment, the use of different oxidants or lower doses of hydrogen peroxide, or to differences in the metabolic activities of the species. In the case of *S. cerevisiae*, it could be related to the higher sensitivity of this species to 0.5mM H_2O_2 in comparison to that of *Candida* sp. This concentration may induce 40% lethality in *S. cerevisiae* and is normally sublethal (95-100% viability) in the case of *Candida* sp.[22,39].

GSH can occur in yeasts in the reduced form (GSH), the oxidized form (GSSG), and as different mixed disulfides, for example GS-S-CoA and GS-S-Cys[13]. The H_2O_2 (0.5 mM/1h treatment) used to induce oxidative stress was probably detoxified in part through the action of the enzyme glutathione-peroxidase (GPx) and the concomitant conversion of GSH into GSSG[29]. Increasing GSSG levels can potentially inhibit protein synthesis in animal and plant cells[40,41], and because of this, *Candida* cells are likely to export GSSG under conditions of oxidative stress, resulting in a decrease in total intracellular glutathione levels.

In yeasts, peroxide resistance has been associated with intracellular GSH levels[42-44]. Further, it has been previously proposed that the rate of removal of H_2O_2 is a direct function of GPx activity × GSH[29]. The highest levels of GSH observed and the intense diminution of intracellular GSH levels in *C. albicans* **(Figure 1),** together with the GPx activities previously observed for this species[20] (Abegg et al. unpublished results), may indicate a more efficient detoxification system of H_2O_2 through GPx/GSH in *C. albicans* than in other *Candida* sp. However, the limitations of the GSH results should be noted, particularly because of the use of 1 isolate of each species, and further comparisons regarding GSH metabolism should be made.

Total antioxidant capacity assays may be broadly classified as electron transfer (ET)-based and hydrogen atom transfer (HAT)-based assays, although these 2 mechanisms may not be differentiated with distinct boundaries in some cases. In fact, most non-enzymatic antioxidant activity (e.g., scavenging of free radicals and inhibition of lipid peroxidation) is mediated by redox reactions. Electron transfer assays include the ABTS, Trolox equivalent antioxidant capacity (TEAC), cupric-reducing antioxidant capacity (CUPRAC), di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium (DPPH), Folin-Ciocalteu, and ferric-reducing antioxidant power (FRAP) methods, each of which use different chromogenic redox reagents with different standard potentials (reviewed in Apak et al.[45]).

The ET mechanism of antioxidant action is based on the following reactions: (1) ROO. + AH/ArOH → ROO^- + $AH.^+$/$ArOH.^+$, (2) $AH.^+$/$ArOH.^+$ + H_2O ↔ A./ArO. + H_3O^+, and (3) ROO- + H_3O^+ ↔ ROOH + H_2O; these reactions are relatively slower than those of HAT-based assays and are solvent- and pH-dependent[45]. Re et al.[46] and Erel[18] developed improved ABTS radical cation decolorization assays using persulfate and hydrogen peroxide, respectively, as the oxidant, and thereby compensated for the weaknesses of the original ferryl myoglobulin/ABTS assay. The 3 TEAC tests developed at different periods, namely the TEAC assay I ($ABTS^{*+}$ generated enzymatically with metmyoglobin and hydrogen peroxide), TEAC II (radical generation with filtration over the MnO_2 oxidant), and TEAC III (with $K_2S_2O_8$ oxidant), are entirely different from each other, are applicable to different solvent media, and their findings for a given antioxidant can vary significantly. The 'pre-addition technique' as in TEAC I, involving the addition of antioxidants before radical generation, could result in an overestimation of antioxidant capacity, because many substances interfere with the formation of the radical; therefore, TEAC I measures the ability to delay radical formation as well as the scavenging of the radical[45].

The advantages of ABTS/TEAC are reported to be operational simplicity, reproducibility, diversity, and most importantly, flexible usage in multiple media to determine both the hydrophilic and lipophilic antioxidant capacities of physiological fluids, since the reagent is soluble in both aqueous and organic solvent media. Aqueous- and lipid-soluble antioxidants are not separated in the TAC protocol employed; therefore, the combined antioxidant activities of all its constituents, including vitamins, proteins, lipids, glutathione, and uric acid, are assessed[45].

The intra-specific TAC variation found here is in agreement with observed variations in the sensitivities of *C. albicans* isolates to oxidants[47]. However, the TAC results did not correlate (rho = 0.051) with the previously reported sensitivity of *Candida* sp. isolates to oxidative stress[22]. One *C. albicans* isolate (51), 2 *C. guilliermondii* isolates (6260 and 73), and 1 *C. krusei* isolate (6258) showed the highest TAC levels. With the exception of the comparison of *C. guilliermondii* isolate 73 with *C. tropicalis* isolate 55, the isolates cited above exhibited significant differences in TAC levels in comparison to all the other isolates tested ($p < 0.05$).

Lapshina et al.[48] compared differences in the ability to scavenge nitroxide (4-amino-2,2,6,6-tetramethylpiperidinoxy; TEMPO), stable free radicals, and alkoxyl free radicals generated by the decomposition of the free radical initiator 2,2'-azobis-2-methyl-propanimidamide dihydrochloride (AAPH) in *S. cerevisiae* strains defective in catalase and superoxide dismutase and with a decreased level of glutathione. Unlike the results obtained here for *Candida* isolates **(Figure 2)**, *S. cerevisiae* cell homogenates did not show considerable strain-related differences.

The TAC based on the scavenging of ABTS free radicals showed a good correlation with the radiation resistance of the yeasts. According to the authors, the results point to the importance of factors other than antioxidative enzymes and glutathione, in the determination of cellular resistance to ionizing radiation and other types of free-radical stress in *S. cerevisiae*.

Balcerczyk et al.[49] demonstrated that the TAC of cell extracts of *S. cerevisiae* showed a stronger dependence on the thiol content as evidenced by the effect of -SH blocking with n-ethylmaleimide (NEM). TAC measured after 10 s was decreased by 83-90% (in different strains) following thiol modification, while TAC measured after a 1-min reduction of ABTS*+ was decreased by 73-80%. According to the authors, the results indicate that thiol groups are a major contributor to the TAC of *S. cerevisiae* and perhaps of other yeast species. These results demonstrate that in cell extracts, in contrast to extracellular fluids, thiol groups constitute the dominant determinant of total antioxidant capacity, at least in *S. cerevisiae.* Depletion of thiols leads to a decrease in TAC. However, cellular adaptation to oxidative stress may involve the mobilization of mechanisms other than an increase in thiol concentrations. This is especially evident in yeast cells, where strains deficient in antioxidant enzymes show increased values of TAC due mainly to thiol-independent mechanisms. Similarly, the adaptation of yeast to conditions of stationary culture and reoxygenation after growth under anoxia predominantly involves antioxidants other than thiols, as demonstrated by Balcerczyk et al.[49].

Considering the mode of action of the enzymes of the peroxiredoxin (Prx) family, which consists of thiol-dependent peroxidases involved in the removal of various types of hydroperoxides in cells, such as hydrogen peroxide, organic peroxides, and peroxynitrite[50,51], and based on the results described above, these enzymes seem to be critical in determining the TAC of yeast cells. In addition to detoxifying peroxides, specific peroxiredoxins have been shown to act as molecular chaperones and to play roles in regulating hydrogen peroxide-mediated cell signaling events[51]. In *S. cerevisiae*, for example, the steady-state protein level of the peroxiredoxin Tsa1 is 45 times that of Gpx3[52,53]. Tsa1 is also the key peroxidase suppressing genome instability and protecting against cell death in *S. cerevisiae*[54,55]. Furthermore, in *S. cerevisiae*, Demasi et al.[56] demonstrated the importance of cytosolic thioredoxin peroxidase I (cTPxI) and its reductant sulfiredoxin in the protection of cells suffering mitochondrial dysfunction, against H_2O_2-induced death.

In *S. cerevisiae*, there are 5 isoforms of Prx, distributed in different cellular compartments[57]. The 2 most abundant peroxiredoxins in these species are Ahp1 and Tsa1[58]. The *TSA1* gene is present in *C. albicans*, *C. glabrata*, *C. tropicalis,* and *C. dubliniensis* and is similar to the *TSA1* and *TSA2* of *S. cerevisiae*. *AHP 11*, *AHP 12,* and *AHP 13* are genes from strain SC5314 of *C. albicans* and show similarity to the *S. cerevisiae* alkyl hydroperoxide reductase *AHP1* (YLR109W) [59].

Urban et al.[60] reported the identification of Tsa1p, a protein that is differentially localized to the cell wall of *C. albicans* in hyphal cells but remains in the cytosol and nucleus in yeast-form cells. According to the authors, this is different from *S. cerevisiae*, where the homologous protein solely has been found in the cytoplasm. These authors reported that *TSA1* confers resistance towards oxidative stress in addition to being involved in the correct composition of hyphal cell walls. Shin et al.[61] also observed that the protein Tsa1p codified by this gene was indispensable in the yeast-to-hyphal transition when *C. albicans* was cultured under oxidative stress. In *C. albicans*, the genes *AHP1* and *HSP12* are regulated by the response regulator gene *SSK1*, and those genes are associated with cell wall biosynthesis and adaptation to oxidative stress[62]. Therefore, it seems that the peroxiredoxin system is critical for the functioning of the antioxidant system of *Candida* and is one of the most important contributors to the TAC in *Candida* cell lysates.

As far as we are aware, this is the first attempt to use a single test of TAC in *Candida*. The use of a single marker of antioxidant capacity has drawbacks and these data must be interpreted with caution. According to Young[17], these single markers measure predominantly low molecular weight and chain-breaking antioxidants, excluding the contributions of antioxidant enzymes and metal binding proteins. The fact that the TAC results did not correlate with the sensitivity of *Candida* sp. isolates to oxidative stress has been reported previously[22] (Abegg et al. unpublished results); the fact that it also did not correlate with total intracellular GSH levels in untreated (rho = 0.042) and treated (rho = 0.058) samples may indicate that a single marker cannot provide a picture of the antioxidant capacity of a *Candida* sp.

Fekete et al.[30] searched for *C. albicans* isolates that are naturally resistant to oxidative stress but did not find this phenotype. They argued that the selection of mutants that are tolerant to oxidative stress *in vivo* would be beneficial to the pathogen-phagocyte interaction, but would be unlikely because of the concomitant and disadvantageous changes in virulence attributes, like morphological transitions and phospholipase secretion. They also point out that an over-efficient antioxidative defense system may be disadvantageous for *C. albicans* by hindering the ROS-triggered activation of genomic ageing and cell death programs that promote adaptation to stresses in the human body. Besides the unlikely selection of *C. albicans* mutants that are naturally oxidant-resistant, certain species like *C. dubliniensis*, *C. guilliermondii*, and *C. famata* are probably not evolutionarily prepared to cope with the first line of immune defense and oxidative stress, even in moderately immunocompromised individuals. This would be reflected in the relative incidence of this species as a causal agent of invasive candidiasis.

Macrophages and neutrophils use ROS, reactive nitrogen species (RNS), and chlorine species for host protection[6-9,63], but the idea that ROS exert direct *in vivo* effects in the fungal killing of *Candida* and other species is still controversial. Balish et al.[64] studied the deficient production of ROS and RNS in mice using gastrointestinal *Candida* inoculation. Although these mice died, an exaggerated immune response rather than an overwhelming fungal infection appeared to cause death. Further, an *in vitro* study with phagocytes from normal and ROS/RNS-deficient mice revealed equal abilities of both to kill *C. albicans*. Wellington et al.[65] considered these data to be in agreement with their results of *C. albicans* suppression of ROS production in phagocytes. However, it seems to be clear that *Candida* species have distinct capacities to deal with oxidative stress, and the inhibition of specific antioxidant molecules may be therapeutically useful in the future.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABSTRACT IN PORTUGUESE

Níveis de glutationa e capacidade antioxidante total em células de *Candida* sp. expostas a estresse oxidativo causado por peróxido de hidrogênio

Introdução: A capacidade de suportar o estresse oxidativo imposto por fagócitos parece ser crítica para que espécies de *Candida* causem candidíase invasiva. **Métodos:** Para melhor caracterizar a resposta ao estresse oxidativo (REO) de oito *Candida* sp. clinicamente relevantes, um componente vital do balanço redox intracelular, a glutationa, foi mensurada pelo método de reconversão DTNB-GSSG redutase e a capacidade antioxidante total (CAT) foi mensurada por um método modificado baseado na descoloração do ABTS*+. Ambos os métodos foram utilizados em extratos celulares das espécies de *Candida* tratadas ou não com peróxido de hidrogênio (0,5mM). **Resultados:** O estresse oxidativo induzido pelo peróxido de hidrogênio claramente reduziu os níveis intracelulares de glutationa. Esta diminuição foi mais intensa em *C. albicans* e os níveis de glutationa em células não tratadas foram também maiores nesta espécie. A capacidade antioxidante total demonstrou variação intraespecífica na capacidade antioxidante. **Conclusões:** Os níveis de glutationa não se correlacionaram com a capacidade antioxidante total mensurada, apesar desta ser a defesa antioxidante intracelular não-enzimática mais importante. Os resultados indicam que a medição isolada da CAT não fornece um quadro claro da habilidade de certa espécie de *Candida* responder ao estresse oxidativo.

Palavras-chaves: *Candida* sp. Glutationa. Capacidade antioxidante total.

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