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Original Article

Evaluation of cell wall damage by dimethyl sulfoxide in *Candida* species

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Abstract

Studies dealing with the response of microorganisms to oxidative stress require the dissolution of oxidant agents in an appropriate solvent. A commonly used medium is dimethyl sulfoxide, which has been considered as an innocuous polar solvent. However, we have observed significant differences between control, untreated cells and those receiving increasing amounts of the oxidant and hence increasing amounts of DMSO, to the maximum allowed of 1%. Here we show that, while this solvent does not influence yeast cell viability, it does affect expression of cell wall proteins as well as catalase activity. Therefore, its use in future studies of oxidative stress as an innocuous solvent should be reconsidered.

Keywords: *Candida* species; Dimethyl sulfoxide; Protein damage; Cell wall and carbonylated proteins

1. Introduction

Candida species are opportunistic pathogens that colonize tissues and organs of immunocompromised and hospitalized patients [1]. To do so, these organisms express a number of virulence and pathogenicity factors that include adhesins, cell wall proteins (CWPs), secretion of lytic enzymes, dimorphism, formation of biofilms and oxidative stress resistance (OSR) [2–4]. OSR is viewed as a relevant factor, since the first line of defense the pathogens confront is that of reactive oxygen species (ROS) generated during the respiratory burst in human phagocytes [5]. A number of in vitro studies have been carried out to understand how these pathogens neutralize ROS and evade the host immune system in vivo [6–11]. Oxidant agents such as hydrogen peroxide (H_2O_2), menadione ($O_2^{\bullet -}$), paraquat, cumene hydroperoxide and diamide, among others, are of common use [7,12,13]. Against this background, we investigated how some pathogenic species of *Candida* respond to different ROS, using oxidants that are poorly soluble in water and other hydrophilic media and highly soluble in hydrophobic solvents such as DMSO, an aprotic compound widely used in studies of oxidative stress as an innocuous dissolvent [14,15]. Yet, during the last decade some reports indicate that DMSO can cause oxidative stress both in yeast and mammalian cells [16,17]. In previous studies, we observed that control yeast cells incubated with different concentrations of DMSO exhibited differences in terms of certain enzymatic activities [2,18]. Therefore, we considered it important to evaluate the effect of this solvent on *Candida* yeast cells by determining its effect on viability, damage to CWPs, protein carbonylation and catalase activity. Results indicated that DMSO does not affect yeast cell viability or protein carbonylation, though it does alter expression of CWPs and activity of catalase, an enzyme involved in OSR.

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2. Materials and methods

2.1. Strains and culture conditions

The strains of *Candida albicans*, *Candida glabrata*, *Candida krusei* and *Candida parapsilosis* used in this study were clinical isolates that were cultured for 48 h at 28 °C on yeast peptone medium (YP; yeast extract, 1%; peptone, 2% glucose). When needed, 2% agar was added to solidify the media [19].

2.2. Assay of DMSO susceptibility

Cultures of *Candida* species grown for 48 h at 28 °C were diluted to an OD_{600nm} of 0.5 in sterile deionized water. Cell suspensions were then incubated with increasing concentrations of DMSO between 0 and 6.0 M in a final volume of 5.0 mL, and incubated with shaking at 28 °C. After 90 min, exponential dilutions were done in 96-well plates and cells were spotted on YPD plates incubated at 28 °C. After 48 h, growth was inspected [6]. This experiment was carried out five times.

2.3. Assay of catalase activity

Yeast cells in stationary phase of growth (48 h/28 °C) exposed or not to DMSO (0, 0.84 and 6.0 M) were used to determine catalase activity according to vendor instructions (Catalase assay kit; Cayman Chemical). Enzyme activity was normalized to total protein in the lysate and expressed in units. One unit is defined as the amount of catalase required for degradation of 1.0 μmol of H_2O_2 per minute.

2.4. Assay of protein carbonylation

The assay of carbonyl groups provides a convenient technique for detecting and quantifying the oxidative modification of proteins. Control and DMSO-treated yeast cells were used to determine the level of protein carbonylation according to the vendor instructions (Protein carbonyl colorimetric assay kit; Cayman Chemical).

2.5. Extraction of CWPs

CWPs were extracted from cultures of *Candida* cells exposed or not to DMSO. For this purpose, cultures were centrifuged at 1300× *g* for 5 min, supernatant was carefully discarded and the cell pellet was resuspended in 2 mL of cold sterile water containing 1 mM phenylmethylsulfonyl fluoride (PMSF), an inhibitor of serine proteases and acetylcholinesterase [2,8,18,20,21]. Cells were disrupted with an ultrasonic homogenizer (Fisher Scientific) at 80% of pulser, 30 W, for alternate 30-s periods of breakage and cooling in a salt ice-water bath until 3 min of breakage were completed. The lysate was centrifuged at 4 °C for 10 min at 13000× *g*, supernatant was discarded and the cell wall pellet was washed exhaustively with cold sterile water containing 1 mM PMSF until a clear supernatant was obtained [8,20]. To extract CWPs, the washed CWPs were resuspended in 2% SDS and 5% β-mercaptoethanol and boiled for 4 min. After removing insoluble material by centrifuging at 6800× *g* for 10 min, the supernatant containing the extracted proteins was collected and the concentration was determined by the DC method (Bio-Rad).

2.6. Two-dimensional gel electrophoresis (2D-PAGE)

CWPs (100 μg) were solubilized in lysis buffer (7 M urea, 2 M thiourea, 2% CHAPS, 65 mM dithiothreitol (DTT), 2% ampholytes pH 3–10, and bromophenol blue) and loaded on 7 cm pH 4–7 strips (Bio-Rad). Isoelectric focusing was done using an Ettan IPGphor (GE Healtcare) as previously described [2,8,18]. After separation by isoelectric focusing, the strip was equilibrated with DTT (10 mg/mL) and CWPs were separated in the second dimension in 12% SDS polyacrylamide gel using a Mini-PROTEAN Tetra Cell (Bio-Rad). Finally, the CWP profile was visualized after staining the gel with colloidal Coomassie blue [8].

2.7. Image analysis of 2D-PAGE gels and protein identification

Analysis and image identification of the 2D-PAGE gels were carried out using PD Quest 7.0 software (Bio-Rad).

2.8. In-gel digestion

Spots of interest were manually excised from Coomassie-blue-stained 2D electrophoresis gels [22] and gel pieces were destained and enzymatically digested according to the modified protocol of Shevchenko et al. [23]. The resulting tryptic peptides were analyzed as described elsewhere [24]. The generated spots were analyzed with a MALDI-TOF/TOF 4800 Plus mass spectrometer (ABSciex, Framingham MA, USA). MS/MS spectra were obtained by fragmentation of selected precursor ions using collision-induced dissociation (CID) and acquired by 3000 shots with a laser intensity of 4400.

2.9. Protein identification

Protein database searches were performed using Mascot 2.3 (Matrix Science) against *Candida*. Mass tolerance for precursor and fragment ions was set at 10 ppm and 0.5 Da, respectively. The enzyme specified was trypsin, and two missed cleavages were allowed. Cysteine carbamidomethylation was specified as a fixed modification and methionine oxidation as a variable modification.

2.10. Statistic analyses

Data were analyzed with two-way ANOVA followed by a Bonferroni posthoc test ($\alpha = 0.05$, *:$P < 0.05$, **:$P < 0.01$, ***:$P < 0.001$). This was carried out with the GraphPad Prism program (Graphpad, USA).

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