HIGH LEVELS OF CATALASE IN SOD MUTANTS OF *SACCHAROMYCES CEREVISIAE* IN HIGH AERATION CONDITIONS

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ABSTRACT

Saccharomyces cerevisiae mutants deficient in superoxide dismutase genes (*sod1Δ*, *sod2Δ* and *sod1Δsod2Δ* mutants) in a stationary phase of growth under high aeration conditions were subjected to H_2O_2 stress. All the mutants were sensitive after H_2O_2 treatment. Glutathione peroxidase levels were significantly lower in *sod1Δ* and *sod2Δ* single mutants than in the wild-type without treatment. After exposure to H_2O_2 concentrations, glutathione peroxidase levels were increased in *sod1Δsod2Δ* double mutants and the *sod2Δ* single mutant, while *sod1Δ* maintained lower gluthatione peroxidase activities. The *sod2Δ* mutant demonstrated a similar catalase activity to that of the wild-type without treatment, whilst decreased catalase activity was observed in conditions of low aeration. Untreated *sod1Δsod2Δ* double mutant cells presented a lower catalase activity. Catalase levels were higher under high aeration conditions than under microaerophilic conditions, including in *sod1Δsod2Δ* cells that contain less H_2O_2, since SOD catalyzes the cleavage of superoxide producing H_2O_2 and oxygen. We suggest that catalase is not essential for sod mutants under normal conditions, but plays an important role in the acquisition of tolerance to oxidative stress induced by high aeration.

Key words: catalase, superoxide dismutase, high aeration, hydrogen peroxide, *S. cerevisiae*

INTRODUCTION

The reduction of oxygen to water supplies the necessary energy for the several functions of our organism. The incomplete reduction of oxygen to water during respiration, however, leads to the formation of redox-active oxygen species (ROS) such as the superoxide anion radical ($O_2{}^\bullet$), the hydroxyl radical ($^\bullet OH$) and hydrogen peroxide (H_2O_2) (2). Studies suggest that mitochondrial respiration is a major source of ROS in vivo, as has been shown *in vitro*, and that these species are produced even under low aeration (7). These ROS have been shown to cause critical damage to DNA, protein and cellular membranes (6).

To defend the organism against ROS, the cell possesses antioxidant enzymes such as glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD). Catalase, known to be one of the most efficient enzymes, reacts with H_2O_2 to form water and molecular oxygen. Glutathione peroxidase removes hydroperoxides (ROOH and H_2O_2) by coupling its reduction to H_2O with oxidation of reduced glutathione (GSH). Superoxide dismutase removes superoxide by converting it to peroxide that can, in turn, be destroyed by CAT or GPx reactions (10).

Saccharomyces cerevisiae, like most other eukaryotes, contains CuZnSOD (the product of the SOD1 gene) in the cytosol, nucleus, and lysosomes and MnSOD (the product of the SOD2 gene) in the mitochondrial matrix.

MnSOD is believed to be the major means of protection against mitochondrial superoxide (4). The sources of ROS relevant to CuZnSOD are less clear because of the location of this enzyme within the cytosol. Neither MnSOD nor CuZnSOD is strictly essential; however, the loss of CuZnSOD has dramatic phenotypic consequences in yeast. Yeast strains lacking CuZnSOD (*sod1Δ*) show reduced growth rates in glycerol and

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ethanol, auxotrophy for lysine and methionine or cysteine, a higher rate of spontaneous mutation and more rapid loss of viability in the stationary phase (3,7,9). *sod2Δ* mutants are oxygen-sensitive and, when required to utilize oxygen, grow poorly. The most severely affected are the *sod1Δsod2Δ* double mutants exhibiting essentially all the characteristics of the single mutant phenotypes.

Growth of a yeast culture consists of three stages. First, there is a period of rapid logarithmic growth, where metabolism is primarily glycolytic. As the glucose is consumed, the culture switches to respiratory growth (known as the diauxic shift) and then to a stationary phase during which energy is generated primarily in the mitochondria. In the stationary phase, yeast stop dividing and exhibit lower metabolic rates, increased antioxidant defenses and are able to survive for weeks to months without additional nutrients (8).

Previous studies performed in our laboratory evaluated the activities of the antioxidant enzymes (CAT and GPx) and the sensibility of the *sod* mutants to H_2O_2 in microaerophilic conditions (9). The highest sensitivity to H_2O_2 was observed in the *sod2Δ* mutant, while the *sod1Δsod2Δ* double mutant was not sensitive. *sod* mutants had lower catalase activity (44%) than wild-type cells, independent of H_2O_2 stress. Untreated cells of *sod1Δsod2Δ* double mutant showed increased glutathione peroxidase activity (126%), while *sod1Δ* had lower activity (77%) than the wild-type. These results suggest that the resistance to H_2O_2 by *sod1Δsod2Δ* cells depends on the induction of glutathione peroxidase and is independent of catalase in stationary phase and microaerophilic conditions (9). Because the level of oxygen modifies the concentration of ROS in the cell, the present investigation intends to evaluate the alterations occurring in the mechanisms of antioxidant defense of the yeast in conditions of high aeration, making a comparison with the results obtained in microaerophilic conditions.

MATERIALS AND METHODS

Strains of *Saccharomyces cerevisiae*

The wild-type *S. cerevisiae* strain, EG103, and the isogenic mutant strains, *sod1Δ*, *sod2Δ* and *sod1Δsod2Δ* were kindly provided by Dr. E. Gralla (University of California, Los Angeles, CA, USA). Disruption of the SOD1 and SOD2 genes was performed as described previously (3,6). The relevant genotypes of the strains are listed in Table 1.

Media and growth conditions

Yeast strains were grown at 30ºC in YPD liquid medium containing 2% glucose, 1% yeast extract and 2% bacto-peptone. The flask volume/medium ratio was 5:1 (high aeration conditions) and flasks were shaken at 200 rpm. For solid medium, 2% agar was added (12). The cell lines lacking CuZnSOD were continually monitored for suppressor activity (3,7).

Table 1. Yeast strains used in this study.

Yeast Strain	Genotype	Ref.
EG103	(DBY746) *MATa leu2-3, 112 his3Δ1 trp1-289 ura3-52 GAL+*	3
EG118	EG103 with *sod1Δ::URA3*	3
EG110	EG103 with *sod2Δ::TRP1*	6
EG133	EG103 with *sod1Δ::URA3 sod2Δ::TRP1*	6

Hydrogen peroxide treatment

Yeast cells were grown to the stationary phase (1-2 x 10^8 cell/mL) in YPD medium at 30ºC. Cells were harvested and washed in sterile saline (0.9% NaCl) and the cell pellets were resuspended in saline and treated with increasing concentrations of H_2O_2 (0.5-5.0 mM) at 30ºC for 1h.

For dose-response curves, aliquots of cells were diluted in saline and plated in triplicate onto YPD to obtain viable counts after 3-5 days of growth at 30ºC.

Enzyme activities

Crude extracts were prepared by glass beads lysis as follows: cells were suspended in lysis buffer (50 mM Tris, 150 mM NaCl, 50 mM EDTA, and 0.2 mM phenylmethylsulfonyl fluoride pH 7.2) with an equal volume of acid washed 425-600 µm glass beads, vortexed for 10-15 cycles (30s each), followed by 30s of cooling. The mixture was then microcentrifuged for 2 min to remove the cellular debris and glass beads (7).

CAT activity was determined spectrophotometrically by monitoring the disappearance of H_2O_2 at 240 nm (13). GPx activity was determined by monitoring the NADPH consumption rate at 340 nm (11). Protein concentration was determined by the Bradford assay (1).

Statistical analysis

Results are reported as means ± SD and were analyzed by the Student's *t* test. Values of P<0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

Wild-type and sod null mutant cultures in the stationary phase of growth under high aeration conditions were treated with increasing levels of H_2O_2 for 1h at 30ºC under non-growth conditions (Fig. 1). All the mutants were sensitive to the treatment with H_2O_2. The *sod2Δ* single mutant and the *sod1Δsod2Δ* double mutant were the most sensitive in relation to the isogenic parent strain. Under microaerophilic conditions, the sensivity of the *sod1Δsod2Δ* double mutant was indistinguishable from that of the wild type (9).

Evaluating the CAT activity (Fig. 2), it was demonstrated that without treatment the *sod2Δ* mutant showed similar CAT activity levels to those of the wild-type. In contrast, under low aeration conditions decreased CAT activity was observed in simple and double mutants (Table 2). The *sod1Δsod2Δ* double mutant, without treatment, presented the lowest levels of CAT, maintaining these low levels after the treatment with H_2O_2 in high aeration (Fig. 2). Under microaerophilic conditions (9), the CAT activity profile was similar, with the *sod1Δsod2Δ* double mutant presenting the lowest enzymatic induction (5 mM H_2O_2, 11.7%). Conversely, the *sod2Δ* single mutant, after H_2O_2 treatment in high aeration, demonstrated the CAT highest activity compared to the wild-type (Fig. 2). A different result was observed in the *sod1Δ* mutant, which initially presented a lower enzymatic activity to that of *SOD+*, but after treatment demonstrated similar levels of induction to those of the wild-type (Fig. 2). Interestingly, the CAT levels under high aeration were much higher than under microaerophilic conditions (Table 2), suggesting that this modification in the aeration level, during the growth of the yeast, induces an increase in the H_2O_2 concentration. Surprisingly, the highest CAT level was also observed in the *sod1Δsod2Δ* double mutant (188% in the control), although this level was significantly lower than that of the wild-type (278% in the control). *sod1Δsod2Δ* double mutant cells contain less H_2O_2 (5) since SOD catalyzes the cleavage of superoxide, producing H_2O_2 and oxygen. With regard to GPx activity, the *sod1Δsod2Δ* double mutant, without treatment, presented similar enzymatic levels to those of the wild-type (Fig. 3, Table 1). After the treatment, this mutant presented the highest GPx activity in comparison with the same untreated cells (1.0 and 5.0 mM H_2O_2). After H_2O_2 treatment, GPx levels were significantly increased when exposed to 0.5-1.0 mM H_2O_2 in the *sod2Δ*, while *sod1Δ* maintained lower GPx activities. However, the high levels of enzymatic activity are not capable of providing these mutants

Figure 1. Sensitivity of *sod* null mutants to H_2O_2. The strains used were: ♦ *SOD+*, ■ *sod1Δ*, ▲ *sod2Δ* and x *sod1Δsod2Δ*. Data represent the mean ± SD of three independent experiments.

with resistance against H_2O_2, since they were affected in the sensibility test.

Evaluating the GPx activity without treatment, it was demonstrated that the enzymatic levels are much greater under high aeration conditions, as also seen with the CAT enzymatic levels (Table 2). The CAT levels were, on average, 200% greater under high aeration, whilst the activity of GPx was increased by 80% compared to the microaerophilic conditions values. The oxidative stress in stationary cultures of yeast may be the consequence of the improved availability of oxygen to the cells, due to a decreased metabolism. Metabolically active cells intensively consume oxygen under conditions of limited aeration, which may lead to low oxygen concentrations within the cells. In stationary cultures, the metabolism is considerably attenuated and, as such, the oxygen concentration within cells may be higher. One-electron reactions of autoxidation-prone cellular components may produce superoxide and, in turn, other reactive oxygen species. The increase in the levels of antioxidant enzymes in yeast entering the stationary phase may, therefore, constitute an adaptative response to the enhanced oxidative damage. In this study, the increase in CAT and GPx activities may also represent a stress response generated by high aeration. These results suggest that catalase is not essential for yeast cells under normal conditions of aeration in these cells, while GPx activities are more important under low aeration conditions.

Table 2. U/mg protein values of CAT and GPx in microaerophilic and high aeration conditions (mean ± SD).

Mutant	Microaerophilic aeration*		High aeration	
	CAT	GPx	CAT	GPx
SOD+	32.2 ± 4.6	26.8 ± 1.6	89.6 ± 4.3 [a]	52.6 ± 1.4 [a]
sod1Δ	15.0 ± 3.2 [b]	21.2 ± 0.4 [b]	59.2 ± 0.3 [ab]	48.3 ± 3.0 [a]
sod2Δ	22.2 ± 2.6 [bc]	30.3 ± 1.4 [bc]	86.6 ± 2.4 [ac]	49.8 ± 1.7 [a]
sod1Δsod2Δ	14.3 ± 2.6 [bd]	34.2 ± 0.5 [bcd]	26.9 ± 1.9 [abcd]	54.3 ± 6.3 [a]

* Manfredini *et al.*, 2004 (9); [a] different from the same strain in microaerophilic aeration, $P < 0.05$; [b] different from SOD+ in the same aeration condition, $P < 0.05$; [c] different from *sod1Δ* in the same aeration condition, $P < 0.05$; [c] different from *sod2Δ* in the same aeration condition, $P < 0.05$; [d] different from *sod1Δsod2Δ* in the same aeration condition, $P < 0.05$.
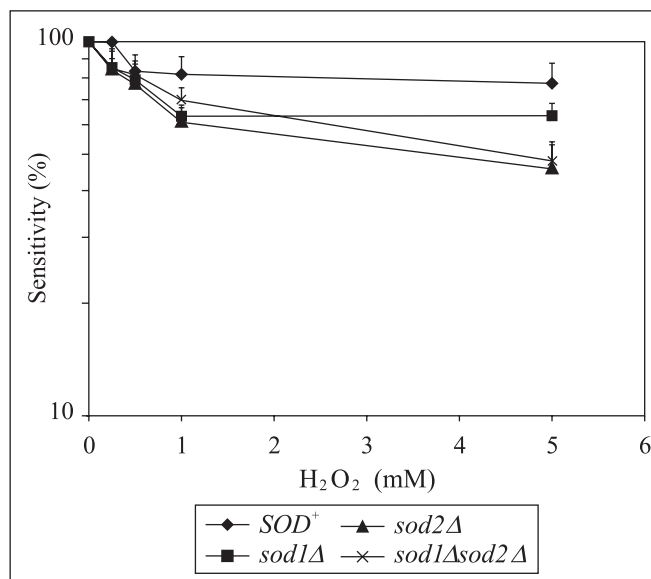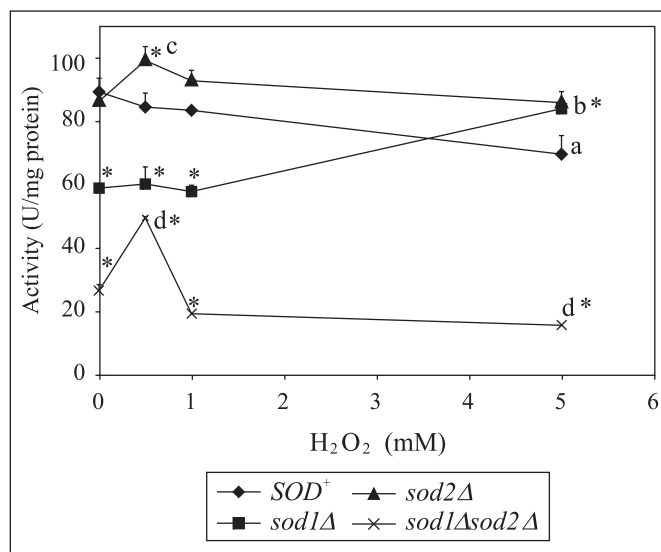
Figure 2. Activity of CAT in wild-type cells ans *sod* mutant cells exposed to H_2O_2. Cells in the stationary phase were treated with the indicated concentrations fo H_2O_2 in sterile saline (0.9% NaCl). For the catalase activity, the rate of decrease in H_2O_2 absorbance at 240 nm was measured. The strains used were: ♦ SOD^+, ■ *sod1Δ*, ▲ *sod2Δ* and ✗ *sod1Δsod2Δ*. Data represent the mean ± SD of three independent experiments. [a] different from SOD^+ untreated cells, p<0.05; [b] different from *sod1Δ* untreated cells, p<0.05; [c] different from *sod2Δ* untreated cells, p<0.05; [d] different from *sod1Δsod2Δ* untreated cells, p<0.05; * different from SOD^+ in the same point.
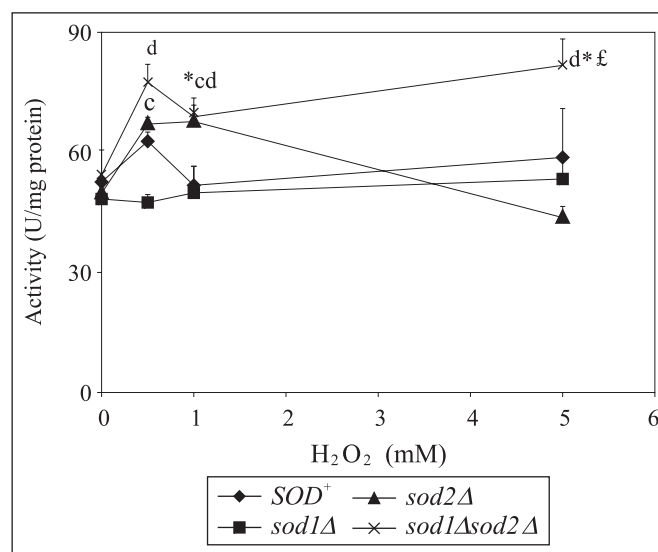
Figure 3. Activity of glutathione in wild-type cells ans *sod* mutant cells exposed to H_2O_2. Cells were treated as described in the legend to Fig. 3. For the determination of glutathione peroxidase activity, the rate of NADPH oxidation was followed at 340 nm. The strains used were: ♦ SOD^+, ■ *sod1Δ*, ▲ *sod2Δ* and ✗ *sod1Δsod2Δ*. Data represent the mean ± SD of two independent experiments. [a] different from SOD^+ untreated cells, p<0.05; [b] different from *sod1Δ* untreated cells, p<0.05; [c] different from *sod2Δ* untreated cells, p<0.05; [d] different from *sod1Δsod2Δ* untreated cells, p<0.05; * different from SOD^+ in the same point, p<0.05; [£] different from *sod1Δ* treated cells with 5 mM H_2O_2, p<0.05.

RESUMO

Altos níveis de catalase em mutantes *sod* de *Saccharomyces cerevisiae* em condições de alta aeração

Saccharomyces cerevisiae deficientes nos genes da superóxido dismutase (mutantes *sod1Δ*, *sod2Δ* e *sod1Δsod2Δ*) cultivados em fase estacionária sob condições de alta aeração foram submetidos ao estresse com peróxido de hidrogênio (H_2O_2). Todos os mutantes mostraram-se sensíveis após o tratamento com o H_2O_2. A enzima glutationa peroxidase (GPx) apresentou níveis significativamente mais baixos nos simples mutantes *sod1Δ* e *sod2Δ* que na cepa selvagem sem tratamento. Após, a exposição a diferentes concentrações de H_2O_2, os níveis da glutationa peroxidase aumentaram no duplo mutante *sod1Δsod2Δ* e no simples mutante *sod2Δ*, enquanto o mutante *sod1Δ* manteve baixa atividade da glutationa peroxidase. O mutante *sod2Δ* demonstrou atividade da catalase similar a da cepa selvagem sem tratamento, enquanto foi observado que a atividade da catalase decresceu em condições de baixa aeração. O duplo mutante *sod1Δsod2Δ* apresentou baixa atividade da catalase mesmo sem tratamento. Os níveis da catalase foram maiores em condições de alta aeração do que em condições microaerófilas, inclusive o duplo mutante *sod1Δsod2Δ* contém menos H_2O_2, visto que, a SOD catalisa a clivagem do superóxido produzindo H_2O_2 e oxigênio. Nós sugerimos neste trabalho que a catalase não é essencial para os mutantes sod sob condições normais, mas ela participa de uma importante via na aquisição da tolerância ao estresse oxidativo induzido por condições de alta aeração.

Palavras-chave: catalase, superóxido dismutase, alta aeração, peróxido de hidrogênio, *S. cerevisiae*

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