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Short communication

THE EFFECT OF GROWTH MEDIUM ON THE ANTIOXIDANT DEFENSE OF *Saccharomyces cerevisiae*

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Abstract: We compared the oxidation of dihydrorhodamine 123, glutathione contents and activities of superoxide dismutase (SOD) and catalase for three wild-type strains of *Saccharomyces cerevisiae* grown on media with different carbon sources. The rate of oxidation of dihydrorhodamine 123 was much higher in respiring cells grown on ethanol or glycerol media than in fermenting cells grown on glucose medium. The total SOD activity was highest on glycerol medium and lowest on ethanol medium, while the catalase activity was highest on glycerol medium. The sequence of glutathione content values was: glucose > ethanol > glycerol.

Key words: Yeast, *Saccharomyces cerevisiae*, Reactive oxygen species, Superoxide dismutase, Catalase, Glutathione

INTRODUCTION

The yeast *Saccharomyces cerevisiae* is an extremely versatile and simple model eukaryotic organism. Its versatility stems from it being a facultative anaerobe that can be grown under variable oxygen pressures, including complete anoxia, and from its ability to utilize various carbon compounds, including both

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Abbreviations used: DTNB – 5,5'-dithiobis-(2-nitrobenzoic acid); H_2R 123 – dihydrorhodamine 123; MES – 2-morpholinoethanesulfonic acid; ROS – reactive oxygen species; SOD – superoxide dismutase; YPD medium – 1% yeast extract, 1% Bacto-peptone, 2% glucose, YPG medium, 1% yeast extract, 1% Bacto-peptone,2% glycerol; YPE medium – 1% yeast extract, 1% Bacto-peptone, 3% ethanol

fermentable and non-fermentable substrates as a source of energy. It may be grown on fermentable carbon sources, especially glucose, deriving its energy from glycolysis and making negligible use of its mitochondria, or on non-fermentable substrates like glycerol and ethanol, activating its mitochondrial oxidative metabolism. In the latter case, the mitochondrial respiratory chain is in operation and the production of reactive oxygen species can be expected to be significantly augmented as an unavoidable by-product of oxidation, assuming the mitochondria are the main cellular sources of superoxide [1-3]. The control of yeast metabolic pathways is complex [4-8], involving not only the induction/repression of enzymes directly active in the metabolism of the appropriate substrates, but also affecting the level of antioxidants and antioxidant enzymes.

There is little data in the literature on the effect of the growth phase and a change in the carbon source on the antioxidant barrier in various strains of *S. cerevisiae*. Although there have been many studies on the effects of xenobiotics and various types of stress on the antioxidant defense of this yeast [6, 9-14], to the best of our knowledge, there has yet to be a study with a simple comparison of the level of activity of the two most important antioxidant enzymes, i.e. superoxide dismutase and catalase, and the main cellular antioxidant, glutathione, in various yeast strains grown under exactly the same conditions. Various wild-type strains used in laboratories often differ in their genetic backgrounds, and can react in a different manner to the same stimuli, such as to a change of carbon source. This study aimed to compare the activities of superoxide dismutase and catalase, and the content of glutathione in three different wild-type laboratory strains of this yeast grown on different carbon sources. In particular, we were interested whether an increased formation of reactive oxygen species can be demonstrated in cells grown on non-fermentable carbon sources, and, if so, how this increase affects the antioxidant defense of yeast cells.

MATERIALS AND METHODS

Strains, media and growth conditions

The following wild-type laboratory strains of yeast were used in this study: SP-4 (MATa leu1 arg4), obtained from Professor T. Biliński (University of Rzeszów, Poland), YPH250 (MATa trp1 his3 leu2 lys2 ade2 ura3), obtained from Professor. Y. Inoue (University of Kyoto, Japan), and ABC154 (MATa ura3 lys2 his3 leu2 ade2 trp1), obtained from Professor A. K. Bachhawat (Institute of Microbial Technology, Chandigarh, India).

The yeast was grown aerobically on YPD liquid medium (1% yeast extract, 1% Bacto-peptone, 2% glucose) and YPG (2% glycerol instead of glucose) or YPE (3% ethanol instead of glucose) media, at 27ºC, with shaking. Yeast cells in the logarithmic phase (A_{600}~0.6) were used.

Dihydrorhodamine oxidation

The level of oxidants able to oxidize dihydrorhodamine 123 (H_2R 123) was estimated fluorimetrically. Cells were washed with the incubation buffer (10 mM 2-morpholinoethanesulfonic acid **(**MES), 0.1 M KCl, 0.14 M NaCl, pH 6.5) containing 2% glucose, and then resuspended in this buffer to a concentration of 10^7 cells/ml. H_2R 123 was added to a final concentration of 5 μM from a stock solution in ethanol. After a 10-min incubation at ambient temperature, the fluorescence intensity of the oxidation product (rhodamine 123) was measured at excitation/emission wavelengths of 504 nm/534 nm, respectively, in a Perkin-Elmer LS-5B spectrofluorimeter.

Preparation of cell extracts

The cells were washed twice with incubation buffer, resuspended in 10 mM MES containing protease inhibitors (protease inhibitor cocktail tablets, Boehringer, Mannheim) and disrupted with 0.46-mm diameter glass beads at 4ºC for 1 hour. Then the cell homogenate was centrifuged (10,000 *x g*, 5 min) and the supernatant was used for the assays.

Biochemical assays

The activity of the catalase in the cell extracts was measured by monitoring H_2O_2 decomposition at 240 nm [15]. The reaction mixture contained 1.9 cm^3 of 50 mM potassium-phosphate buffer, pH 7, and 100 mm^3 of the cell extract. One unit of catalase activity corresponds to the amount of enzyme which destroys 1 μmol of H_2O_2 per minute, yielding a decrease in absorbance equal to 0.0145 units per minute.

The superoxide dismutase (SOD) activity was measured by the inhibition of adrenalin autoxidation [16]. The reaction mixture contained 100 mm^3 of 10 mM adrenalin in 10 mM HCl, 50 mM carbonate buffer, pH 10.2, and 20, 30 or 40 mm^3 of the cell extract in a total volume of 3 cm^3. The increase in absorbance due to adrenalin autoxidation was monitored at 480 nm and the percentage inhibition of the maximal rate of increase in absorbance (0.025/min) was determined. One unit of superoxide dismutase activity corresponds to the amount of the enzyme which inhibits the rate of adrenalin autoxidation by 50%.

Total glutathione content was measured via the recycling assay with glutathione reductase, according to Akerboom and Sies [17]. The reaction mixture contained 1 cm^3 of 0.1 M phosphate buffer, pH 7, with 1 mM EDTA, 100 mm^3 of the cell extract, 50 mm^3 of the NADPH solution (4 mg/cm^3 in 0.5% $NaHCO_3$), 20 mm^3 of DTNB (1.5 mg/cm^3 in 0.5% $NaHCO_3$), and a solution of glutathione reductase yielding an activity of 6 units/cm^3. The rate of increase in the absorbance of the nitrobenzoate anion was measured at 412 nm.

The protein content was estimated by the method of Lowry *et al.* [18]. All the results are presented as the mean values ± SD from at least three independent experiments.

RESULTS AND DISCUSSION

The redox physiology of yeast cells is profoundly affected by the source of carbon utilized. Yeast grown on glucose derive energy from fermentation, whereas mitochondrial respiration is triggered on non-fermentable carbon sources [19]. Mitochondria are the main source of the superoxide radical and other ROS derived from this radical, so the production of ROS is expected to be greater in yeast grown on non-fermentable carbon sources. Indeed, the oxidation of dihydrorhodamine 123 (H_2R 123) was considerably higher in yeast grown on ethanol and glycerol media than in yeast grown on the glucose medium (Fig. 1).

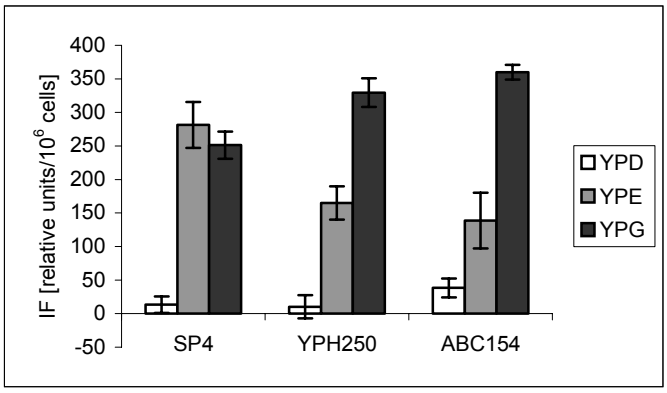

Fig. 1. The effect of the type of medium on the oxidation of dihydrorhodamine 123 by various strains of the yeast *S. cerevisiae* (logarithmic phase). The cells were washed with the incubation buffer containing 2% glucose, and resuspended in this buffer to a concentration of 10^7 cells/ml. H_2R was added to a final concentration of 5 μM. After a 10-min incubation at the ambient temperature, the fluorescence intensity of the oxidation product (rhodamine 123) was measured.

H_2R 123 is known to be oxidized to the fluorescent compound rhodamine 123 by hydrogen peroxide and organic peroxides in reactions catalyzed by peroxidases or ferric ions, but also by a range of other oxidants [20, 21]. Though the identification of specific oxidants of H_2R 123 in a cell is not easy, the rate of oxidation is usually assumed to reflect the overall level of the reactive oxygen species. However, such an interpretation may be too straightforward, since the measured rate of dihydrorhodamine 123 oxidation is a resultant of the rate of ROS production and the level of endogenous antioxidants competing with the probe for the ROS [22, 23].

The growth medium (i.e. carbon source) also had a profound effect on the glutathione content of the yeast cells. A surprising finding was that cells grown on the glucose medium had a considerably higher glutathione content than those grown on the ethanol or glycerol media, in particular on the latter (Fig. 2). This could be due to the need for glutathione in the glyoxalase pathway, although this

question requires further study. The glyoxalase pathway in the fermenting yeast mainly disposes of methylglyoxal, formed as a by-product of glycolysis from triose phosphates, and is dependent on glutathione [24]. Another important parameter, not evaluated in this study, is the redox potential of cellular glutathione, dependent on the concentration of both the reduced and oxidized forms of this compound [25, 26]. The value of this parameter may also be dependent on the source of carbon in the yeast cultures.

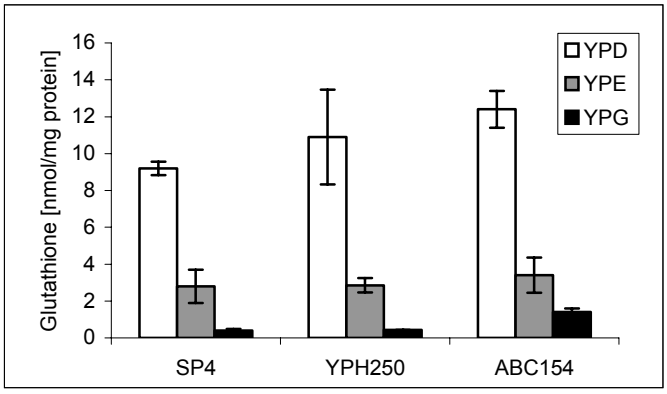

Fig. 2. The effect of the type of medium on the glutathione content of various strains of the yeast *S. cerevisiae*. Cells were grown in YPD (1% yeast extract, 1% Bacto-peptone, 2% glucose), YPE (3% ethanol instead of glucose) or YPG media (2% glycerol instead of glucose) until the mid-logarithmic phase (A_{600}~0.6). The total glutathione was measured via the glutathione recycling assay.

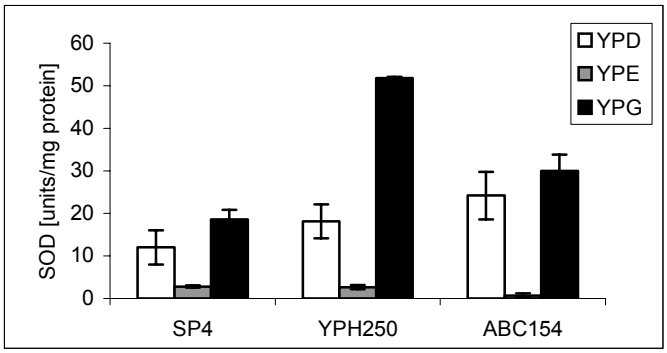

Fig. 3. The effect of the type of medium on the superoxide dismutase activity of various strains of the yeast *S. cerevisiae*. The cells were grown in YPD, YPE or YPG media until the mid-logarithmic phase (A_{600}~0.6). Superoxide dismutase activity was measured by the inhibition of adrenalin autoxidation.

The total superoxide dismutase activity was the lowest in yeast grown on the ethanol medium (Fig. 3), while the catalase activity was the highest in cells grown on glycerol (Fig. 4). The pattern of changes was similar in all the strains, though the absolute values of glutathione content and enzyme activities showed considerable differences. Interestingly, the pattern of changes in glutathione content and catalase activity in response to the type of medium were opposite, in line with the idea of an overlapping defense against oxidative stress by glutathione and catalase [27].

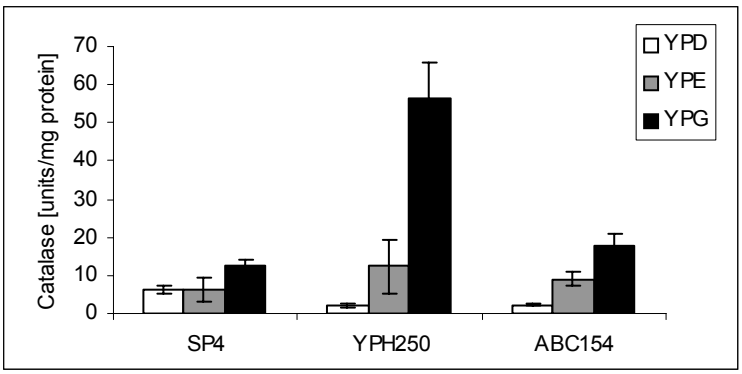

Fig. 4. The effect of the type of medium on the catalase activity of various strains of the yeast *S. cerevisiae.* The cells were grown in YPD, YPE or YPG media until the mid-logarithmic phase (A_{600}~0.6). Catalase activity was estimated by spectrophotometric measurement of the rate of decomposition of hydrogen peroxide.

These results point to considerable differences in the level of antioxidant defense and the rate of oxidation of dihydrorhodamine 123 in yeast grown on different media. Respiratory media, inducing much higher ROS production than the glucose media due to the activation of the mitochondria, increase the catalase activity. The low activity of superoxide dismutase on ethanol medium is hard to explain. It has been reported that upon diauxic shift, the level of expression of both SOD1 (CuZnSOD) and SOD2 (MnSOD) increase, but the level of SOD1 decreases thereafter [6]. Most (ca 90%) of the activity measured in the cell extracts is due to cytoplasmic CuZnSOD, so variations in MnSOD activity, more important in this respect, may remain unnoticed in this assay.

Based on the data here, it seems that the adaptation of the yeast to increased ROS production involves the induction of antioxidant enzymes rather than an increase in the glutathione level. Taking into account the energetic costs of synthesis and the reduction in glutathione content, as compared with the cost-free function of superoxide dismutase and catalase, once synthesized, such a solution seems less expensive in terms of energy requirements.

A comparison of the rate of dihydrorhodamine 123 oxidation (Fig. 1) and the content of glutathione (Fig. 2) and catalase activity (Fig. 4) demonstrates an

inverse relationship between the rate of oxidation of an exogenous fluorogenic probe and the level of glutathione, which may support the view of the importance of scavenging of ROS by endogenous antioxidants for the yield of their detection [22, 28].

The results obtained demonstrate significant differences in the rate of dihydrorhodamine 123 oxidation and levels of glutathione and in the activities of the antioxidant enzymes in these three wild-type yeast strains, grown under the same conditions. The reasons for the interstrain differences are difficult to explain without detailed knowledge of the genetic and resultant metabolic differences between the strains; however, they should be kept in mind when comparing data reported for various yeast strains and should be considered in metabolomic studies.

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