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INVOLVEMENT OF CATALASE IN SACCHAROMYCES CEREVISIAE HORMETIC RESPONSE TO HYDROGEN PEROXIDE

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Abstract. In this study, we investigated the relationship between catalase activity and H_2O_2-induced hormetic response in budding yeast *S. cerevisiae*. In general, our data suggest that: (*i*) hydrogen peroxide induces hormesis in a concentration- and time-dependent manner; and (*ii*) the effect of hydrogen peroxide on yeast colony growth positively correlates with the activity of catalase that suggests the enzyme involvement in overall H_2O_2-induced stress response and hormetic response in yeast.

Keywords: yeast, hormesis, hydrogen peroxide, reproductive ability, catalase.

1. INTRODUCTION

Hormesis is a phenomenon of particular interest in biology, medicine, pharmacology and toxicology. It has been observed in a variety of organisms: from bacteria to humans, responding to a wide range of chemical, physical, and biological stressors, including ionizing radiation, chemotherapeutic agents, metal ions, pesticides, antibiotics, ethanol, aldehydes, chloroform, pro-oxidants, hypergravity, and so forth [10, 20, 25, 26]. The specificity of stress response is determined by the nature of stressor, intensity/duration of its action, and physiological state of an organism. According to the hormesis theory, low doses of stress-inducing factors lead to stimulatory hormesis response and improvement of cellular and organism functions, whereas at high doses the deleterious effects prevail [19, 21]. Interestingly, hormesis may activate defense pathways ensure protection against higher doses of the same agent ("pre-adaptation") as well as other specific stressors ("cross-protection") [5, 28, 30]. Therefore, hormetic response suggests the existence of complex mechanisms, which sense and respond to different kinds of stress.

Recent studies strongly support the notion that hydrogen peroxide plays a dual role in biological systems [1, 6, 17, 27]. Its effect can be considered as either beneficial or harmful, because at high concentrations H_2O_2 causes oxidative damage to cell structures, whereas at low concentrations it is a part of many cellular signaling systems. At low concentrations hydrogen peroxide also plays a crucial role in the induction of hormesis [1, 17, 24, 28].

Manipulation of reproductive potential through hormesis-stimulating compounds, like hydrogen peroxide, appears to be an effective approach to improve yeast survival and cross-adaptation to different kinds of stress. In our recent study, it has been demonstrated that hormetic concentrations of hydrogen peroxide caused yeast cross-resistance to severe stress induced by high concentrations of

ethanol, acetic and propionic acids [28]. The global-stress transcription factors Msn2/4p and Yap1 have been found to play an important role in the hormetic effects by low concentrations of hydrogen peroxide and involved in yeast cross-adaptation by low concentrations of hydrogen peroxide [2, 28]. Since catalase is a member of H_2O_2-stimulon regulated by the Msn2/4p and Yap1 transcriptional factors in *S. cerevisiae* [14, 18, 22, 29], the enzyme could be a key element of either the hormetic stress response to hydrogen peroxide.

Here, we used model organism *S. cerevisiae* to study the effect of different concentrations of hydrogen peroxide on cellular reproductive ability and potential role of catalase in H_2O_2-induced hormetic response.

2. METHODS AND MATERIALS

The *Saccharomyces cerevisiae* strains used in this study were YPH250 (wild type *MATa trp1-Δ1 his3-Δ200 lys2-801 leu2-Δ1 ade2-101 ura3-52*) and its isogenic derivative YWT1 (YPH250 *ctt1Δ::URA3 cta1Δ::TRP1*) described earlier [16]. The strains were kindly provided by Prof. Yoshiharu Inoue (Kyoto University, Japan). Chemicals were obtained from Sigma-Aldrich Chemical Co. (USA) and Fluka (Germany). All chemicals were of analytical grade.

Yeast cells were grown with shaking at 175 r.p.m. and 28 °C in Erlenmeyer flasks containing YPD liquid medium (1% yeast extract, 2% peptone, and 2% glucose) in a volume that respected the ratio 1 : 5 regarding media volume to flask volume. For experiments, overnight cultures were diluted to about 10^6 cells/mL in YPD. Cells from experimental cultures after 24 h growth were split into two portions: one used for the reproductive ability evaluation and another used for cell-free extract preparation and catalase activity measurement.

Yeast reproductive ability was determined in yeast collected by centrifugation (5 min, 8000 g), washed with 50 mM potassium phosphate (K-phosphate) buffer (pH 7.0), resuspended in the same buffer. Aliquots of the suspension (10^8 cells/mL) were exposed to different concentrations of hydrogen peroxide followed by their incubation at 28 °C for different time periods as described earlier [4]. Control cells were incubated under the same conditions but without hydrogen peroxide. Yeast reproductive ability was analyzed by plating in triplicate on YPD agar after proper dilution. The plates were incubated at 28 °C for 3 days and the colony forming units (CFU) counted [11]. Reproductive ability was expressed as percentage of total amount of cells plating on YPD agar.

The activity of catalase was measured in cell-free extracts prepared from yeast treated with different concentrations of hydrogen peroxide for different periods of time in YPD medium at 28 °C as described earlier [3, 31]. Control cells were incubated under the same conditions but without hydrogen peroxide. Then yeast were collected by centrifugation (5 min, 8000 g), washed with 50 mM K-phosphate buffer (pH 7.0). The yeast pellets were resuspended in lysis buffer (50 mM K-phosphate buffer (pH 7.0), 1 mM phenylmethylsulfonyl fluoride and 0.5 mM EDTA). Cell extracts were prepared by vortexing yeast suspensions with glass beads (0.5 mm) as described earlier [3, 31] and kept on ice for immediate use. Catalase activity was measured spectrophotometrically with a SF-46 spectrophotometer (LOMO, USSR). The enzyme activity was determined by monitoring the disappearance of hydrogen peroxide at 240 nm using the extinction coefficient for hydrogen peroxide of 39.4 $M^{-1}cm^{-1}$ [3, 31]. One unit of catalase activity was defined as the amount of supernatant protein that utilized 1 μmol of substrate per minute. The activity was measured at 25 °C and expressed per milligram of soluble protein in supernatant.

Protein concentration was determined by the Coomassie brilliant blue G-250 dye-binding method [7] with bovine serum albumin as the standard. Experimental data are expressed as the mean value of four to eight independent experiments ± the standard error of the mean (SEM), and statistical testing was carried out used Student's t-test.

3. Results and Discussion

Oxidative stress, depending on its intensity, can be considered as either harmful or beneficial [10, 19, 20, 21, 23]. Mild oxidative stress has been found to stimulate microorganisms' biological functions and resulted in an acquisition of their resistance to high doses of the same as well as other stressors [17, 23, 28]. An improvement of cellular and organismic functions by moderate stress is known as the phenomenon of hormesis, that may be graphically represented by the biphasic dose-response dependence, characterized by the stimulation in the low dose zone, followed by an inhibitory response at higher doses [10, 20]. Hormetic stimulatory response is usually limited to the 30–60% increase in a biological function under mild stress conditions [8, 9, 10]. Among many stress-induced agents, hydrogen peroxide has been found to play a crucial role in the induction of hormesis [17].

The findings of the present study also demonstrate typical biphasic concentration-response curve, exhibiting hormetic effect of hydrogen peroxide (Fig. 1). The reproductive ability of yeast cells, that was determined as colony forming units (CFU), was assessed after the 60-min incubation period with different H_2O_2 concentrations. The concentrations lower than 0.15 mM H_2O_2 did not affect reproductive ability. The peak hormetic response of yeast cells was observed at 0.15 mM H_2O_2. At the hormetic concentration of hydrogen peroxide, yeast showed about 160% of the control (without H_2O_2) colony growth of yeast. At higher concentrations (0.2-2.5 mM H_2O_2), reproductive activity decreased to almost control value, and the parameter dropped to 73-55% of the initial reproductive ability during yeast incubation with as high as 10-100 mM H_2O_2.

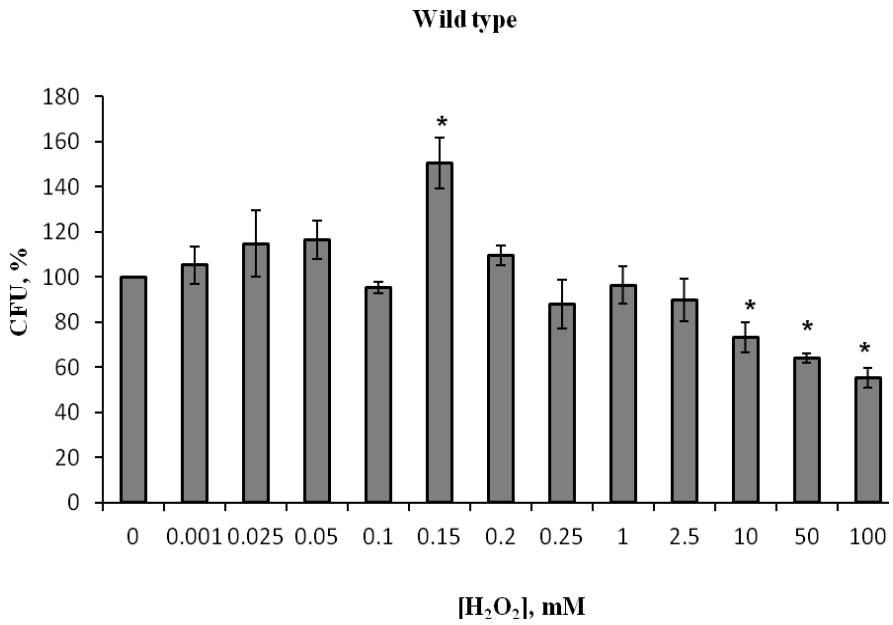

*Fig. 1. Effect of hydrogen peroxide on reproductive ability of S. cerevisiae YPH250 (wild type). Results are shown as the mean ± SEM (n = 4-10). *Significantly different from control (without H_2O_2) with P < 0.05.*

The data presented in Fig. 1 are consistent with previous studies reported stimulatory effect of low concentrations of hydrogen peroxide (≤0.4 mM H_2O_2) and its toxicity at the concentrations higher than 0.5 mM H_2O_2 in *S. cerevisiae* [13, 28]. Stimulation of the reproductive potential through hormesis-induced compounds, like H_2O_2, appears to be an effective approach to improve yeast survival and cross-adaptation to different stresses. Earlier we demonstrated that, unlike ethanol, hormetic concentrations of hydrogen peroxide caused yeast cross-resistance to different kinds of severe stress [28].

Molecular mechanisms of hormesis due to H_2O_2-induced stress are yet to be elucidated, but there are some, which can be suggested to involve catalase, a member of the H_2O_2-stimulon in *S. cerevisiae* [14, 22, 29]. Fig. 2 demonstrates the effect of different concentrations of hydrogen peroxide on the activity of catalase in yeast. The activity increased significantly (by 1.6-fold) during incubation with as

little as 25 μM H_2O_2 and reached a maximum content (about 2-fold higher than control) in the presence of 50-100 μM H_2O_2. In previous studies, the activation of catalase by low concentrations of hydrogen peroxide has been associated with a cytosolic CTT1 isoenzyme [3, 14]. Fig. 2 shows also that at higher H_2O_2 concentrations (0.15-2.5 mM H_2O_2), catalase activity decreased to control value, and dropped significantly by 2.1-4.8-fold comparing to control during yeast incubation with the highest concentrations used (10-100 mM H_2O_2, respectively). Lower catalase activity in cells exposed to 10-100 mM H_2O_2 could be explained by inactivation of catalase *in vivo* by high concentrations of the substrate. Our previous experiments also demonstrated that catalase activity did not depend linearly on hydrogen peroxide concentration, and results were interpreted from the point of view of enzyme inactivation by high peroxide concentrations [3, 31].

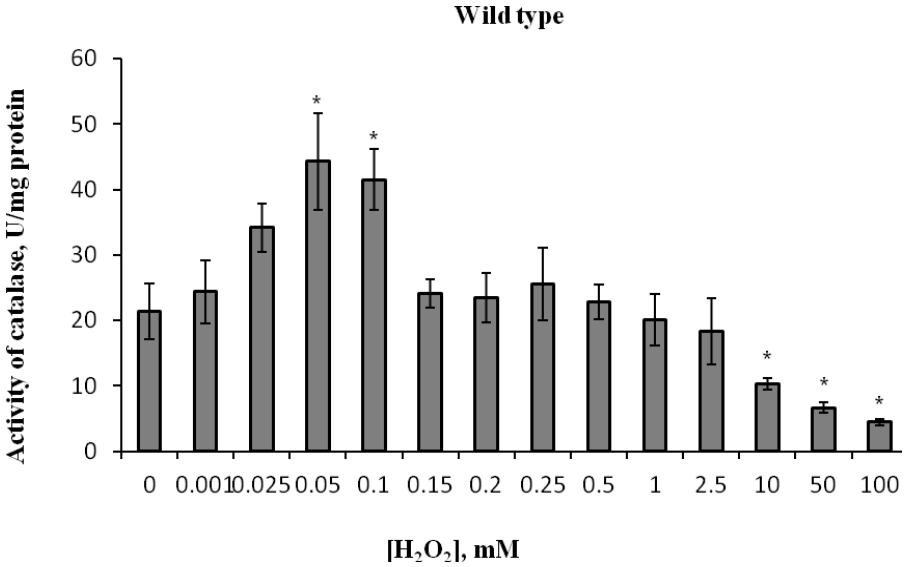

*Fig. 2. Effect of hydrogen peroxide on the activity of catalase in S. cerevisiae YPH250 (wild type). Results are shown as the mean ± SEM (n = 5-8). *Significantly different from control (without H_2O_2) with P < 0.05.*

Figure 3 shows the time course of the reproductive ability of yeast cells stressed by exposure to 0.15 (the hormetic concentration) and 100 mM H_2O_2 (the highest concentration used). As seen from the Figure the parameter did not change in the control cells over the 120-min incubation period without hydrogen peroxide. In the case of yeast treatment with 0.15 mM H_2O_2, the parameter gradually rose reaching the highest values on 40-120 min (~2.5-fold higher than zero time control). Incubation of yeast with 100 mM H_2O_2 over the 60-120-min incubation period caused 2.5-fold reduction of the reproductive ability as compared with zero time control. The activity of catalase rose over time in cells treated with 0.1 mM H_2O_2 (Fig. 4). A significant increase in the enzyme activity of 1.7-fold higher than zero time control was noted after 60 min incubation, but it significantly reduced over the 120-min incubation period and became virtually the same as that in control. At the same time yeast exposure to 1 mM H_2O_2 caused decrease in catalase activity over time (2-fold lower after 120 min than zero time control). Control experiments (without H_2O_2) showed that the activity did not change over the 120-min incubation period. Thus from the presented above data an important role of catalase in H_2O_2-induced hormetic response of yeast can be supposed. This suggestion is confirmed by the results demonstrated in Fig. 5. As seen in the Figure, hydrogen peroxide did not affect the reproductive ability of mutant yeast cells lacking catalase activity.

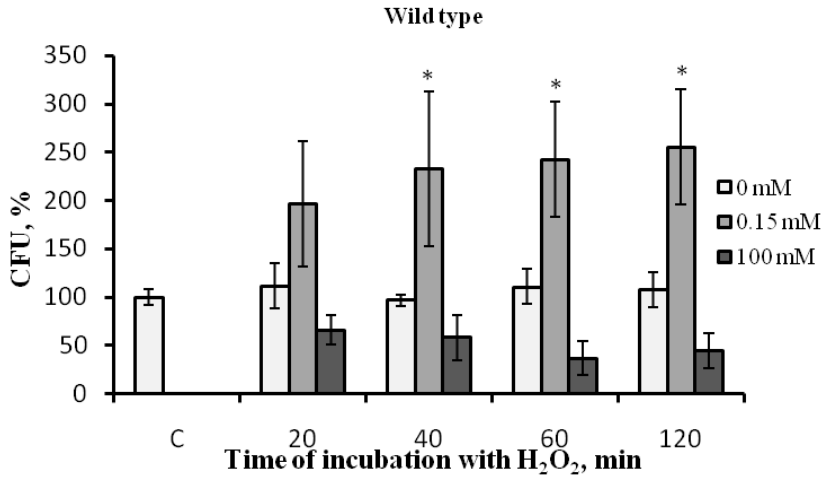

*Fig. 3. The time course of changes in reproductive ability of S. cerevisiae YPH250 (wild type). Results are shown as the mean ± SEM (n = 4-8). *Significantly different from control (without H_2O_2) with P < 0.05.*

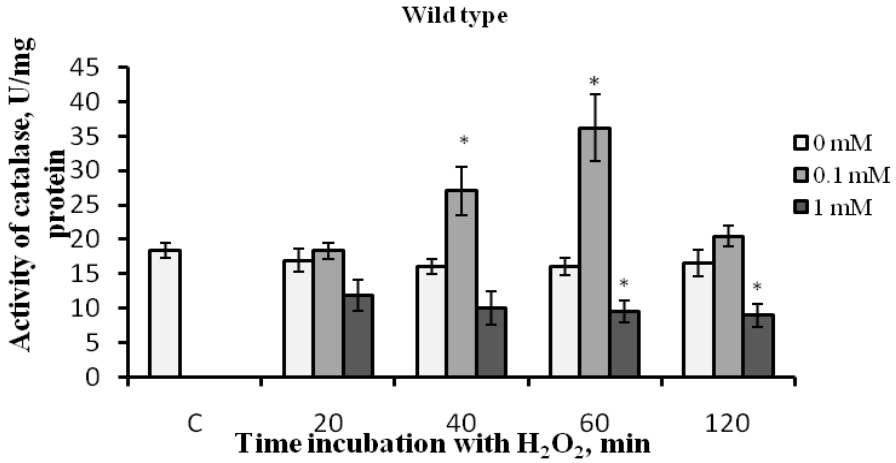

*Fig. 4. The time course of changes in catalase activity in S. cerevisiae YPH250 (wild type). Results are shown as the mean ± SEM (n = 4-8). *Significantly different from control (without H_2O_2) with P < 0.05.*

In general, yeast cells exposed to hydrogen peroxide respond by the reorganization of gene expression at different levels of the flow of the genetic information [18, 22, 29]. The oxidative stress response in yeast has been analyzed in detail at the genome, transcriptome, proteome and post-proteome level. However, many studies reported a lack of correlation between the expression of certain genes at different levels of cellular organization [12, 15, 29]. The weak elevation of catalase activity in yeast treated with sublethal concentrations of H_2O_2 reported earlier [3, 4, 16] as well as found in this study (2-3-fold higher than control), does not correspond to high level of the respective enzyme molecules (15-fold higher than control) [14]. It is well known that exposure of microorganisms to low sublethal concentrations of hydrogen peroxide may lead to the acquisition of cellular resistance to a subsequent lethal stress [13, 28, 30]. Earlier we supposed that yeast cells exposed to low concentrations of hydrogen peroxide respond accumulating stress-protectant molecules [4, 31]. Since a sudden increase in antioxidant activities could dramatically disturb the intracellular redox homeostasis, it seems most of the respective protein molecules synthesized *de novo* remain non-active. This provides the cells with the capability to respond quickly and survive consequent lethal challenge via rapid post-translational activation of these proteins, and catalase in particular.

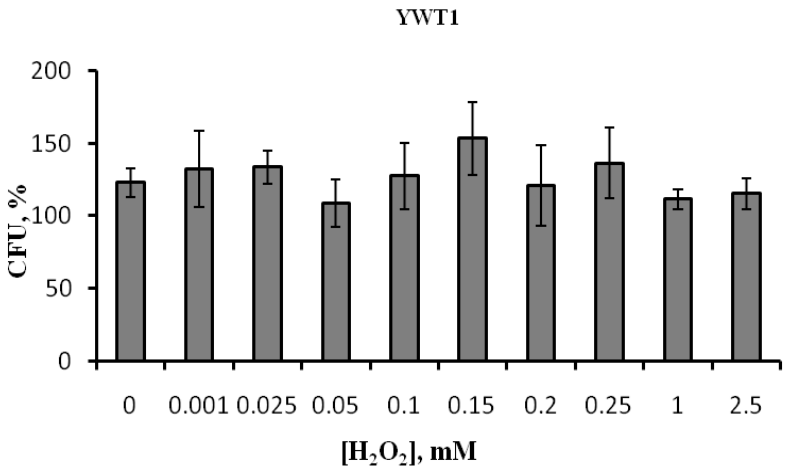

Fig. 5. Effect of hydrogen peroxide on reproductive ability of S. cerevisiae YWT1 (lacking catalase). Results are shown as the mean ± SEM (n = 3-4).

4. CONCLUSIONS

Therefore, considering the literature and present data it can be supposed that moderate stress induced by low concentrations of hydrogen peroxide results in catalase-dependent hormesis, the effect that provides organism with a potential to survive consequent lethal stress (Fig. 6). On the other hand, at high concentrations of hydrogen peroxide oxidative processes seem to predominate, therefore cellular proteins and even catalase may be inactivated. In turn, this leads to cell/organism death under further severe stress.

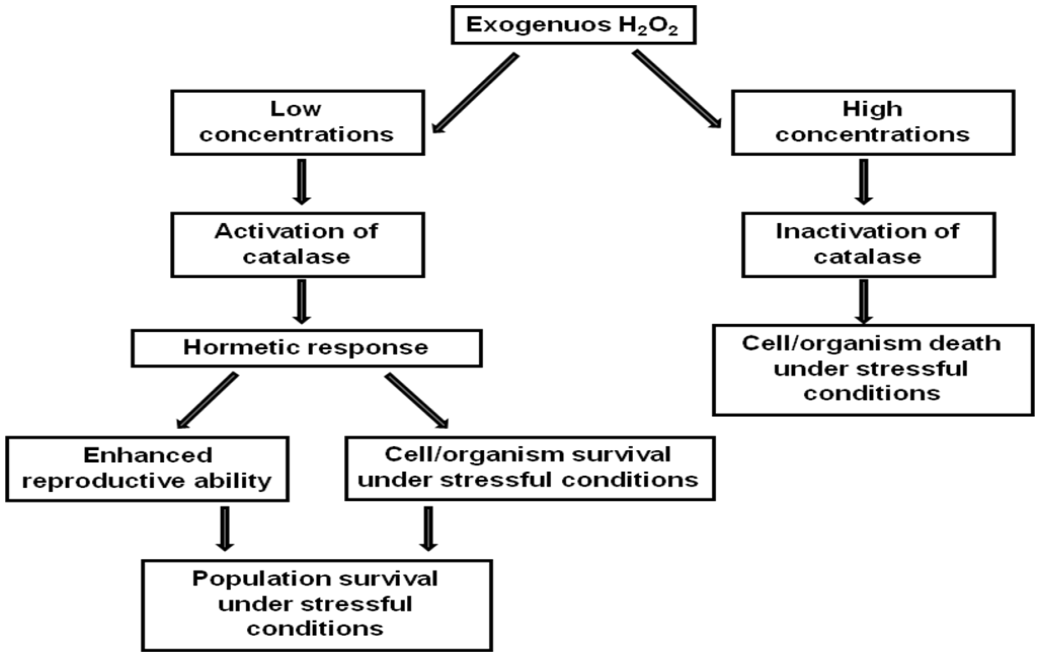

Fig. 6. Concentration-dependent dual effect of hydrogen peroxide in vivo.

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Васильковська Р.А., Бурдилюк Н.І., Семчишин Г.М. Участь каталази в горметичній чутливості *Saccharomyces cerevisiae* до пероксиду водню. *Журнал Прикарпатського університету імені Василя Стефаника*, **2** (1) (2015), 107–114.

Досліджено взаємозв'язок між активністю каталази і горметичною відповіддю *S. cerevisiae* на дію пероксиду водню. Загалом, отримані дані свідчать про те, що: 1) горметичний ефект залежить від концентрації та тривалості інкубації клітин з H_2O_2; та 2) ріст колоній дріжджів за дії пероксиду водню позитивно корелює з активністю каталази, що свідчить про участь ферменту в загальній відповіді на стрес, викликаний H_2O_2, а також в горметичній відповіді дріжджів.

Ключові слова: дріжджі, гормезис, пероксид водню, репродуктивна здатність, каталаза.