EFFECT OF MENADIONE AND HYDROGEN PEROXIDE ON CATALASE ACTIVITY IN *Saccharomyces* YEAST STRAINS

Nadejda EFREMOVA[*], Elena MOLODOI[*], Agafia USATÎI[*], Ludmila FULGA[*], Tamara BORISOVA[*]

[*] *Institute of Microbiology and Biotechnology of Academy of Sciences of Moldova, Chişinău, Moldova*
Corresponding author: Nadejda Efremova, Institute of Microbiology and Biotechnology, 1, Academiei, 2028 MD, Chişinău, Moldova, phone: +373(22)738013, fax: +373(22)725754, e-mail: efremova.nadejda@gmail.com

Abstract. It has been studied the possibility of utilization of two important oxidant factors as regulators of catalase activity in *Saccharomyces* yeasts. In this paper results of the screening of some *Saccharomyces* yeast strains for potential producers of catalase are presented. Results of the screening for potential catalase producer have revealed that *Saccharomyces cerevisiae* CNMN-Y-11 strain possesses the highest catalase activity (2900 U/mg protein) compared with other samples. Maximum increase of catalase activity with 50-60% compared to the reference sample was established in the case of hydrogen peroxide and menadione utilization in optimal concentrations of 15 and 10 mM. This research has been demonstrated the potential benefits of application of hydrogen peroxide and menadione as stimulatory factors of catalase activity in *Saccharomyces* yeasts.

Keywords: *Saccharomyces cerevisiae;* antioxidant enzyme; catalase; oxidants.

INTRODUCTION

In recent years, biomedical studies have demonstrated that oxidative stress is involved in etiology of different diseases such as atherosclerosis, Alzheimer's and Parkinson's disease, myocardial infarction, asthma, skin disorders, etc [9, 11, 25]. Cells contain a number of antioxidant defenses, however the free radical formation can exceed the cell's antioxidant capacity, resulting in oxidative stress. Oxidative stress is the result of an imbalance in pro-oxidant/antioxidant homeostasis that leads to the generation of toxic reactive oxygen species [5, 18]. ROS are highly damaging towards DNA, lipids and proteins [10]. Biological damage caused by oxygen radicals includes oxidization of membrane fatty acids, resulting in lipid peroxidation, oxidization of proteins and DNA damage [25].

It is known that live organisms activate antioxidant enzymes as response to the influence of free radicals [8, 17]. The use of natural antioxidants that neutralize consequences of oxidative stress is of a great interest of many researches. The antioxidant enzymes as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPx) and glutathione reductase (GSHR) serve as primary line of defense in destroying free radicals [12, 23]. Catalase is one of the most efficient antioxidant enzymes. Catalase is a tetrameric enzyme, which consists of four similar subunits, each containing a heme group. Catalase sequentially oxidizes hydrogen peroxide to molecular oxygen and water [20, 23]. This enzyme contributes to prevent the destructive modifications at cellular level, such as DNA breakage.

One of the actual problems of modern biotechnology is the search of new sources of primary material for the elaboration of medical preparations, food additives with a high range of antioxidant action. Recent studies have demonstrated the importance of *Saccharomyces* cerevisiae as eukaryotic model for the study of mechanisms of oxidative stress. The utilization of *Saccharomyces* yeast as the source of antioxidant enzymes presents the perspective for futher investigation [12, 15]. Yeasts have some advantages compared with traditional sources of enzymes with antioxidant effects. The advantages of *Saccharomyces* yeast strains are high grown rate, a short period of cultivation (4-5 days), the relatively low price of the components of nutrient medium. In addition, there is no need to use the biological material of animal origin for catalase obtaining that significantly reduces production costs and reduces the risk of obtaining of infected extracts in the case of utilization of bovine erythrocytes.

In this context presents interest the utilization of two important oxidant factors as regulators of enzymatic activity of yeasts. The capacity of menadione and hydrogen peroxide to promote oxidative stress can be used for the increase in catalase activity.

Relevance of investigations is determined by the wide range of application of catalase due to the high antioxidant activity. Protein extracts on the base of catalase from *Saccharomyces* yeast strains could be used for the obtaining of antioxidant preparations. The aim of the present study was to establish the effect of two oxidants on catalase activity in the yeast *Saccharomyces cerevisiae*.

MATERIALS AND METHODS

Strains. For the investigation were used eight *Saccharomyces* yeast strains from National Collection of Nonpathogenic Microorganims of the Institute of Microbiology and Biotechnology of Academy of Sciences of Moldova under the following registration numbers: baker's yeast - *Saccharomyces cerevisiae* CNMN-Y-11; *Saccharomyces cerevisiae* CNMN-Y-16; brewer's yeast -*Saccharomyces carlsbergensis* CNMN-Y-15; wine yeast *Saccharomyces cerevisiae* CNMN-Y-17; *Saccharomyces cerevisiae* CNMN-Y-18; *Saccharomyces cerevisiae* CNMN-Y-19; *Saccharomyces cerevisiae* CNMN-Y-20; *Saccharomyces cerevisiae* CNMN-Y-21.

Medium. Cultivation of yeasts was effectuated on the nutritive medium YPD with the following

composition (g/L): glucose - 20.0, peptone - 20.0, yeast extract - 10.0 [2]. YPD medium is widely used for *Saccharomyces* yeast studies and it is optimal for investigation of antioxidant enzymes.

Culture conditions. Cultivation was carried out in Erlenmayer flasks capacity 1L, containing 0.2 L of nutritive medium at 180-200 rpm agitation rate, at temperature of 25…27ºC for 120 hours.

The following oxidants were selected as regulators of catalase activity: menadione and hydrogen peroxide. Yeast cells were pretreated with menadione and hydrogen peroxide on the first day of cultivation. The oxidant concentrations were varied from 3 to 30 mM.

The determination of biomass accumulation was effectuated gravimetrically, through the separation from cultural liquid by centrifugation [14].

Protein content was determined spectrophotometrically by Lowry [16].

The determination of catalase activity where the disappearance of hydrogen peroxide is followed spectrophotometrically at a wavelength of 240 nm was effectuated according to Aebi [1].

RESULTS

Primary outcome of interest was to select *Saccharomyces* yeast strain with high catalase activity. Some biologically active yeast strains being deposited in National Collection of Nonpathogenic Microorganims were used in screening for potential producers of catalase. The experimental results regarding the following screening are presented in Figure 1, 2. Initially, it was studied biomass accumulation of eight *Saccharomyces* yeast strains used in investigation (Fig. 1).

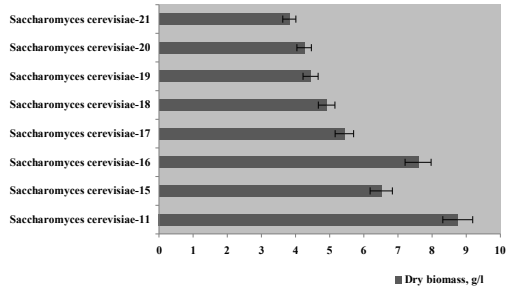

Figure 1. Determination of dry biomass of some *Saccharomyces* yeast strains

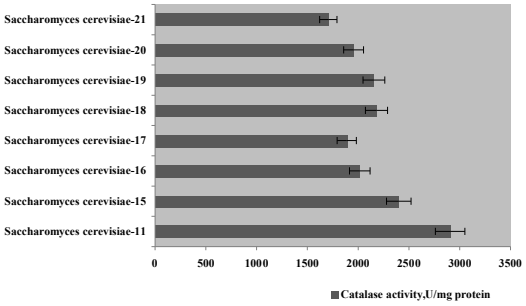

Figure 2. Determination of catalase activity of some *Saccharomyces* yeast strains

This study allowed to evidentiate three strains of yeast of the genus Saccharomyces with high productivity: *Saccharomyces cerevisiae CNMN-Y-11* (8,75 g/L), *Saccharomyces carlsbergensis* CNMN-Y-15 (6,5 g/L) and *Saccharomyces cerevisiae* CNMN-Y-16 (7,59 g/L). Indices of catalase activity at eight *Saccharomyces* yeast strains are presented in Figure 2.

Results of the screening for potential catalase producer of eight yeast strains have demonstrated that *Saccharomyces cerevisiae* CNMN-Y-11 possesses the highest catalase activity (2900 U/mg protein) compared with other samples. It was also determined that another yeast strain *Saccharomyces carlsbergensis* CNMN-Y-15 is characterized by high activity of antioxidant enzyme catalase (2400 U/mg protein). Thus, *Saccharomyces cerevisiae* CNMN-Y-11 yeast strain with higher indices of biomass accumulation and catalase activity among other studied yeast strains was selected for further investigation.

The study of scientific data enabled us to establish the importance of menadione and hydrogen peroxide as factors that can induce oxidative stress at live organisms [13, 24]. Following this, the possibility of using oxidant factors (hydrogen peroxide and menadione) as catalase activity regulators of the *Saccharomyces cerevisiae* CNMN-Y-11 yeast strain selected as a source of enzymes with antioxidant properties was studied. To evaluate cells survival in oxidative stress condition, it was determined yeast productivity. The results of the influence of hydrogen peroxide and menadione on biomass accumulation of *Saccharomyces cerevisiae* CNMN-Y-11 are presented in Figure 3.

Analyzing obtained results it was found that administration of hydrogen peroxide and menadione on first day of cultivation had beneficial effect on biomass accumulation of *Saccharomyces cerevisiae* CNMN-Y-11. The high indices of biomass accumulation (9,57-10,40 g/L) was obtained by using menadione in concentration of 10-15 mM. The administration of hydrogen peroxide to the nutritive medium YPD in concentration of 5-10 mM also contributed to increase of yeast biomass (8,90-9,40 g/L).

Catalase activity in *Saccharomyces cerevisiae* CNMN-Y-11 yeast strain, characterized by increased activity of this enzyme, cultivated in the presence of hydrogen peroxide and menadione was subsequently studied (Fig. 4).

It was established that both hydrogen peroxide and menadione have beneficial effect on catalase activity in *Saccharomyces cerevisiae* CNMN-Y-11. As can be seen from this figure, maximum increase of catalase activity with 50-60% (4500-4605 U/mg protein) compared to the reference sample (2900 U/mg of protein) was established in the case of hydrogen peroxide and menadione utilization in optimal concentrations of 15 and and 10 mM. The obtained results have demonstrated that hydrogen peroxide and menadione can be used as stimulatory factors of catalase activity in *Saccharomyces* yeasts.

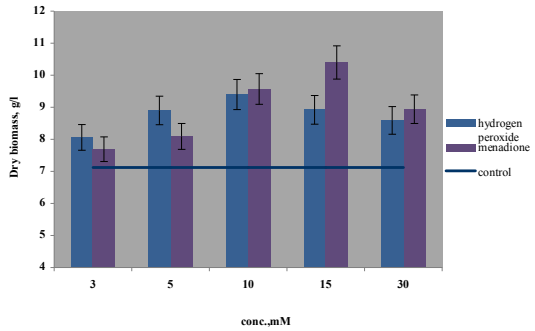

Figure 3. The influence of hydrogen peroxide and menadione on biomass accumulation of *Saccharomyces cerevisiae* CNMN-Y-11

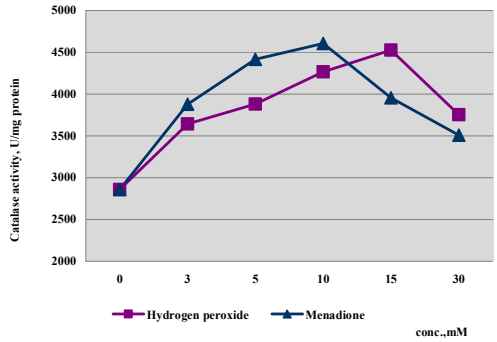

Figure 4. The influence of hydrogen peroxide and menadione on catalase activity in *Saccharomyces cerevisiae* CNMN-Y-11

DISCUSSION

The role of antioxidant enzymes such as catalase, superoxide dismutase, glutathione peroxidase has been a topic of great interest for more than a decade [4, 5, 21, 22]. Catalase is an important antioxidant that functions to detoxify hydrogen peroxide. In general, catalase is one of the key enzymes in the protection of cells against free radicals and induction of catalase by oxidative stress may be the cellular defense and response.

According to the literature data, the ROS - caused oxidative breakage is prevented by antioxidant defences [10, 11, 19]. The mode of yeast adaptation to oxidative stress are not yet completely studied and presents research interest.

The obtained results have demonstrated that the utilization of hydrogen peroxide and menadione at established optimal concentrations contributed to significant increase in catalase activity(4500-4605 U/mg protein) in selected as active catalase producer *Saccharomyces cerevisiae* CNMN-Y-11 yeast strain. Stimulation of catalase activity in microorganisms by induced oxidative stress could be of a great importance for modern biotechnology. Recent investigation have revealed that hydrogen peroxide has the potential to cause widespread cellular damage [3, 6, 24]. It is known that menadione generates reactive oxygen species (ROS) [7, 13]. The formation of ROS can be prevented by increased synthesis of antioxidants, particularly antioxidant enzymes. The increase in catalase activity may represent an adaptive stress response to the enhanced oxidative damage. This response is intended to neutralize cellular damage caused by oxidant factors. The results of the present study suggest that both hydrogen peroxide and menadione can serve as oxidative stress - provoking factors. Thus, the supplementation of nutritive medium YPD with hydrogen peroxide stimulates directly catalase activity. Hydrogen peroxide can serve also as inductor of enzyme activity. The catalase activity can be increased by high substrate concentration (H_2O_2).

The obtained biomass of *Saccharomyces cerevisiae* CNMN-Y-11 yeast strain with high catalase activity could be recommended for the obtaining of new antioxidant preparations of proteic nature which are intended to neutralize the oxidative stress with the following application in the field of medicine, food industry and cosmetology. Catalase can be used for medical preparations elaboration for profilaxy and treatment of diseases associated with oxidative stress (atopic dermatitis, eczema, osteoarthritis, etc.); food supplements for ensuring human organism's resistance against free radicals action, as well as cosmetic products for regeneration and prevention of premature skin aging.

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