THE UTILIZATION OF SOME IRON AND ZINC COMPOUNDS AS REGULATORS OF CATALASE ACTIVITY AT *Saccharomyces cerevisiae*

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Abstract. The main aim of this study was to examine the impact of some zinc and iron compounds as oxidative stress factors on catalase activity, which is known to be important defense system of microorganisms to metal stress. For the investigation was used baker's yeast strain - *Saccharomyces cerevisiae* CNMN-Y-11 previously selected as a source of protein and catalase. The obtained results have revealed that compounds of iron and zinc with citrate and acetate contributes to the accumulation of yeast biomass and have beneficial effect on the catalase activity at selected yeast strain. The maximum increase of catalase activity in yeast biomass was established in case of iron and zinc citrate supplementation to the nutritive medium in optimal concentration of 15.0 mg/l. Results of the present study could be used for the elaboration of new procedures of catalase obtaining by directed synthesis with the utilization of selected metal compounds.

Keywords: yeasts, antioxidant enzyme, *Saccharomyces cerevisiae*, catalase, metal compounds

INTRODUCTION

In the last decades, oxidative stress became a widespread phenomenon involved in the pathology of different diseases and disorders [2, 4, 19]. Oxidative stress can be defined as the physiological changes resulting from the formation of excess quantities of ROS (reactive oxygen species). It is well known that the excess production of ROS can be scavenged by both enzymatic and non enzymatic mechanisms [10]. The study of alternative sources of bioactive substances which possess antioxidant properties is one of the perspective directions of modern biotechnology worldwide. Along with other microorganisms yeasts serve as biotechnological objects for numerous investigations.

The production of *Saccharomyces* yeasts biomass rich in valuable biologically active substances is advantageous due to nontoxic nature and high productivity. Other important aspect is the utilization of culture mediums based on low cost substrates with high yield. *Saccharomyces cerevisiae* is an excellent model system for the molecular study of oxidative stress [21]. In addition, *Saccharomyces* yeasts strains also can be considered a source of antioxidants, as well as antioxidant enzymes, such as catalase, superoxide dismutase, glutathione peroxidase [14]. An important role in cell protection from the negative effect of oxidative stress has antioxidant enzyme catalase (CAT). Catalase is a heme-containing enzyme that catalyzes the dismutation of highly reactive hydrogen peroxide to water and oxygen [24]. Catalase is one of the most efficient antioxidant enzymes known. This enzyme contributes to the prevention of damaging modifications at cellular level, how it would be DNA breakage that leads to different forms of cancer or other chronic degenerative diseases. Catalase, as well as superoxide dismutase, has been reported to be efficacious for vitiligo treatment [15]. Recently, a study on the levels of catalase and superoxide dismutase in skin of vitiligo patients confirmed that these antioxidant enzymes may play an adjuvant role in the management of vitiligo in addition to specific therapies [23]. Even though, recent studies have suggested antiallergical and antiinflamatory properties of catalase [20].

According to the literature data, metals and metal compounds can serve as stimulators of synthesis of biologically active substances. Metals play important roles in a wide variety of biological processes of living systems. Previously, it was established that some compounds of Zn(II) can be used as regulators of wine yeast strains productivity and carbohydrates accumulation [25]. Thus, manganese and zinc were found to be effective stimulators of superoxide dismutase activity at cyanobacterium *Spirulina platensis* [6]. Researchers have demonstrated the possibility of utilization of some compounds of iron and cobalt as regulators of hydrolase biosynthesis at mycelial fungi [5].

Zinc has an important role in metabolism of *Saccharomyces* yeasts because it is incorporated in the structure of proteins involved in the defence antioxidant systems against oxidative stress. Zn is an essential micronutrient involved in wide variety of biochemical processes [16]. One iron characteristics is its potential to undergo redox processes. Iron is known to have structural and regulatory role in many enzymes and other proteins directly. Iron is the prevailing redox metal in biological systems and it is found in a variety of key catalytic sites of many enzymes [26]. The aim of this investigation presents a study of possibility of utilization of some compounds of Fe(III) and Zn(II) compounds as regulators of catalase activity at *Saccharomyces cerevisiae* yeasts.

MATERIALS AND METHODS

Strains. For the investigation was used baker's yeast strain - *Saccharomyces cerevisiae* CNMN-Y-11 from National Collection of Nonpathogenic Microorganims of Institute of Microbiology and Biotechnology of Academy of Sciences of Moldova.

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 [3]. 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 metal compounds were selected as regulators of catalase activity: iron citrate ($FeC_6H_5O_7 \cdot 2H_2O$), iron acetate ($Fe(C_2H_3O_2)_3$), zinc citrate ($Zn_3(C_6H_5O_7)_2 \cdot 2H_2O$) and zinc acetate ($Zn(C_2H_3O_2)_2 \cdot 2H_2O$). These compounds were added to nutrient medium YPD in concentration of 5.0; 10.0; 15.0; 20.0; 30.0 mg/l on the first day of cultivation.

The determination of biomass accumulation was effectuated gravimetrically, through the separation from cultural liquid by centrifugation [13].

Protein content was determined spectrophotometrically by Lowry [18].

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] in modification [7].

RESULTS

Previously undertaken studies enabled to select some metal compounds of iron and zinc which present interes to be used as regulators of catalase activity at *Saccharomyces* yeasts. Thus, *Saccharomyces cerevisiae* CNMN-Y-11 yeast strain previously selected as a source of protein and catalase with higher indices of biomass accumulation and catalase activity was selected for further investigation.

The experimental results regarding the influence of iron and zinc compounds with citrate and acetate on productivity of *Saccharomyces cerevisiae* CNMN-Y-11 and catalase activity are presented below. Initially, it was studied biomass accumulation of selected yeast strain used in investigation in oxidative stress condition due to the administration of chosen metal compounds (Fig. 1-2).

It was established that all tested compounds of iron and zinc have had beneficial effect on biomass accumulation of selected yeast strain. The obtained results have demonstrated higher indices of biomass accumulation in the case of iron citrate administration in concentrations of 10.0 and 15.0 mg/l (with 57-53% compared to control). The administration of iron compound with acetate contributed to the insignificant growth of biomass with the maximum increasing with 30% at concentration of 20.0 mg/l. Further the effect of zinc citrate and acetate on productivity of *Saccharomyces cerevisiae* CNMN-Y-11 was studied (Fig. 2).

The administration of zinc compounds contributes to the growth of yeast biomass with the maximum effect (with 28-38% compared to the control) at the utilization of zinc citrate in concentrations of 5.0-10.0 mg/l. The utilization of zinc acetate had a slightly effect on biomass accumulation. The further increase of concentrations resulted in the decrease of process of biomass accumulation. However, indices of dry biomass exceeded the control value.

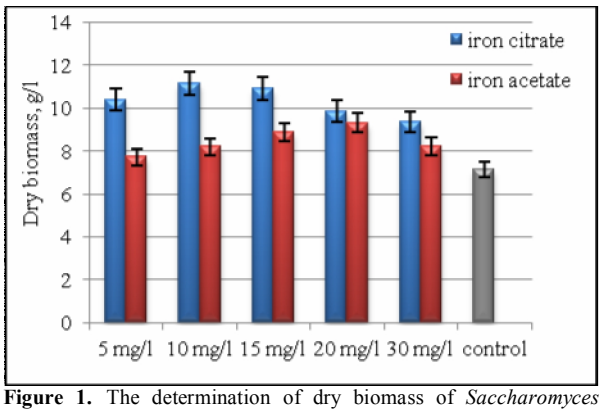

Figure 1. The determination of dry biomass of *Saccharomyces cerevisiae* CNMN-Y-11 cultivated in the presence of iron citrate and acetate

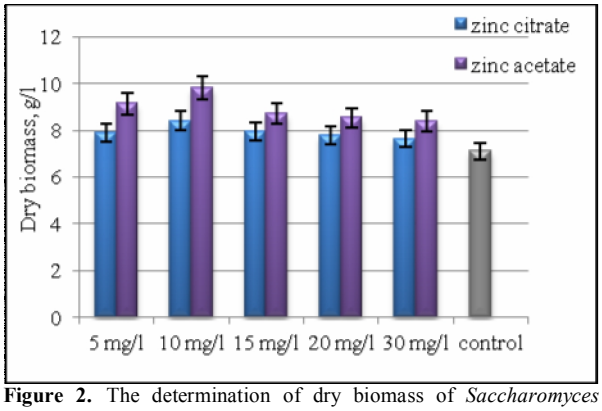

Figure 2. The determination of dry biomass of *Saccharomyces cerevisiae* CNMN-Y-11 cultivated in the presence of zinc citrate and acetate

Then it was determined catalase activity at selected yeast strain cultivated in the presence of iron and zinc compounds Analyzing obtained results it was found that administration of both iron and zinc compounds had significant stimulatory effect on catalase activity at *Saccharomyces cerevisiae* CNMN-Y-11 (Fig. 3-4). The activity of catalase was gradually increased with the metal compound concentration. It can be noted that catalase activity was increased by 108-120% compared to the reference sample in the case of utilization of iron citrate within limits of concentration of 10.0-15.0 mg/l. The subsequent decrease of catalase activity with the increase of concentration up to 30.0 mg/l may be induced by the fact that defense cell mechanism breaks down due to excessive metal concentration.

The selected for investigation zinc compounds also had considerable effect on activity of catalase in yeast biomass (Fig. 4). In this paper it was established that the effect of metal compounds on catalase activity is variable and depends on the metal and concentration. Maximum increase of CAT activity (by 125% compared to the reference sample) was established in the case of utilization of zinc citrate in the concentration of 15.0 mg/l. To a lesser extent than citrate compounds, both iron and zinc acetate contributed to the increase of catalase activity. So, zinc

and iron acetate have stimulated CAT activity by 82.0-90.0% compared to the reference sample in the optimal concentration.

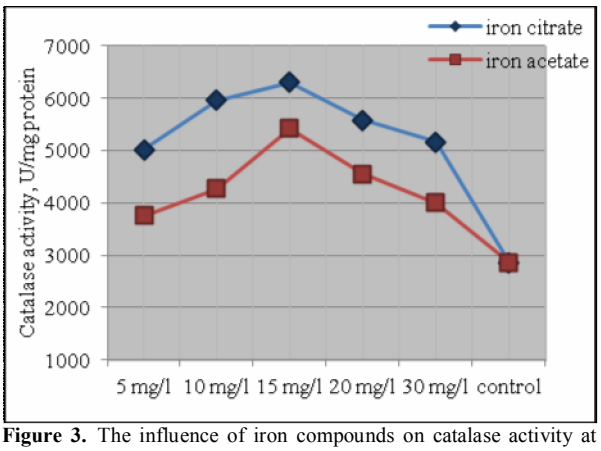

Figure 3. The influence of iron compounds on catalase activity at *Saccharomyces cerevisiae* CNMN-Y-11

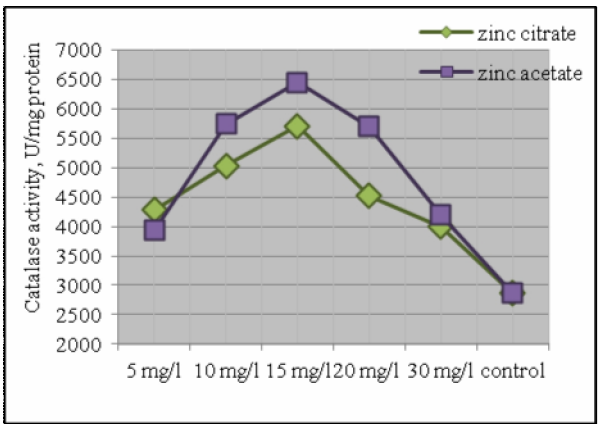

Figure 4. The influence of zinc compounds on catalase activity at *Saccharomyces cerevisiae* CNMN-Y-11

Thus, according to obtained results, can be indicated significant stimulatory effect of selected for investigation iron and zinc compounds on the activity of antioxidant enzyme catalase at *Saccharomyces cerevisiae* strain. This paper has revealed that *Saccharomyces cerevisiae* can serve as object for studying of metal action on the process of growth of yeast strain and synthesis of catalase. The analysis of the influence of iron and zinc compounds with citrate and acetate on the content of other components of antioxidant defense yeast system presents great interest for further investigation.

DISCUSSION

Recent studies demonstrated that metals and metal compounds can serve as potent oxidizing agents [8, 17]. According to the literature data, metals and metal compounds can activate redox pathways that lead to the stimulation of defense antioxidant system [26]. It was confirmed that metals like cadmium increased activities of superoxide dismutase, catalase and peroxidase in arbuscular mycorrhizal fungi [11]. Other study has established that activities of antioxidant enzymes were also high in cadmium-exposed *Saccharomyces cerevisiae* yeast cells [21]. This effect is induced by the fact that cadmium can generate ROS indirectly by depleting the cell's anti-oxidant defenses or by displacing redox-active metals from proteins. But the use of cadmium is generally limited due to its toxicity. Moreover, this metal possess carcinogenic, mutagenic and teratogenic properties under experimental conditions [9]

In spite of this, the effect of iron and zinc compounds with citrate and acetate on catalase activity in *Saccharomyces cerevisiae* has not been studied yet. The obtained data have revealed that *Saccharomyces cerevisiae* is very sensitive to metals action. It was evident that the utilization of selected compounds of iron and zinc in optimal concentrations has a direct effect on the production of reactive oxygen species.

For the first time, it was demonstrated that iron and zinc citrate can be used as effective stimulators of catalase activity in *Saccharomyces* yeasts. Another important factor was the fact that these metal compounds are non-toxic for yeasts and have beneficial action on yeast biomass accumulation within the limits of the chosen concentrations. The significant increase of catalase activity established in this paper can be explained by the metal-mediated formation of free radicals, in particular, highly reactive hydrogen peroxide. The supplementation of nutritive medium YPD with compounds of iron and zinc stimulates directly catalase activity. Hydrogen peroxide can serve as inductor of enzyme activity. So, the catalase activity can be increased by high substrate concentration (H_2O_2). Thus, metal excess provokes oxidative stress that results in concomitant activation of catalase. Can be proposed, that the possible mechanism, involved in the activation of catalase due to the action of metal compounds on yeast cells, includes the formation of new molecules of this enzyme, which contributes to the reduction of hydroxyl radical formation from H_2O_2. Thus, the results indicate that the tolerance of *Saccharomyces cerevisiae* CNMN-Y-11 strain to different metal compounds was correlated with the reactive oxygen species generation in the cell and with the efficiency of antioxidant enzyme catalase.

Furthermore, the stimulatory action of iron citrate can be induced by the fact that the activity of catalase depends on iron ions - the most effective cofactor of this enzyme. The obtained data are similar with the results of other researchers which have established the possibility of copper ions to enhance activity of Cu, Zn-SOD at *Yarrowia lipolytica* yeasts [12].

In conclusion, these results can be used for the elaboration of new procedures of catalase obtaining by directed synthesis with the utilization of selected metal compounds. The stimulation of catalase activity at *Saccharomyces* yeasts by induced oxidative stress could be of a great importance for modern biotechnological investigations. Finally, the present study has demonstrated that obtained biomass of *Saccharomyces cerevisiae* CNMN-Y-11 strain with enhanced activity of catalase enzyme with antioxidant properties can be proposed for further elaboration of

food supplements, medical remedies for prophylaxis and treatment of diseases, caused by the negative consequences of oxidative stress on live organisms and, also, for the elaboration of cosmetic preparations for the protection against solar radiation and treatment of skin diseases.

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REFERENCES

[1] Aebi, H., (1984): Catalase in Vitro. Methods in Enzymology, 105: 121-126.
[2] Ames, B., Shigenaga, M., Hagen, T., (1993): Oxidants and Antioxidants, and the degenerative diseases of aging. Proceedings of the National Academy of Sciences of the United States of America, 90: 7915-7922.
[3] Anghel, I., Vassu, T., Segal, B., (1991): Biologia şi tehnologia drojdiilor. Bucharest: Tehnica, vol. 2, 386 p.
[4] Cooke, M., Evans, M., Dizdaroglu, M., Lunec, J., (2003): Oxidative DNA damage: mechanisms, mutation, and disease. The Journal of the Federation of American Societies for Experimental Biology, 17(10): 1195-1214.
[5] Deseatnic-Ciloci, A., Clapco, S., Stratan, M., Tiurin, J., Labliuc, S., Coropceanu, E., Bologa, O., Bulhac, I., Ciobanica, O., (2009): Influenţa unor dimetilglioximaţi ai Fe(II) şi Co(II) asupra biosintezei hidrolazelor la fungi miceliali. 33 Congress American-Romanian Academy of Arts and Sciences, Sibiu, Montreal, Quebec, 2: 409-412.
[6] Efremova, N., Bulimaga, V., Reva, V., Rudic, V., Chiriac, T., Gulea, A., Zosim, L., Elenciuc, D., Djur, S., Bivol, C., (2012): The influence of some metallocomplexes on phycocianin content and superoxide dismutase activity in biomass of *Spirulina platensis* (NORDST.) Geitler (Cyanophyta). Algologia, 22(3): 250-257.
[7] Efremova, N., Usatîi, A., Molodoi, E., (2013): Method of determination of catalase activity. Patent 4205 MD, BOPI, 2.
[8] Ercal, N., Gurer-Orhan, H., Aykin-Burns, N., (2001): Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage. Current Topics in Medicinal Chemistry, 1(6): 529-539.
[9] Flora, S., (2009): Metal Poisoning: Threat and Management. Al Ameen Journal of Medical Science, 2(2): 4-26.
[10] Flora, S., (2007): Role of free radicals and antioxidants in health and disease. Cell Molecular Biology, 53(1): 1-2.
[11] Garg, N., Bhandari, P., (2012): Influence of cadmium stress and arbuscular mycorrhizal fungi on nodule senescence in *Cajanus cajan* (L.) Millsp. International Journal of Phytoremediation, 14(1): 62-74.
[12] Hiroyasu, I., Masahiro, I., Hiroshi, T., Masanori, J., (2007): Characteristics of copper tolerance in *Yarrowia lipolytica*. BioMetals, 20: 773-780.
[13] Hong-Zhi, L., Qiang, W., Xiao-Yong, L., Sze-Sze, T., (2008): Effects of spaceflight on polysaccharides of *Saccharomyces cerevisiae* cell wall. Applied Microbiology and Biotechnology, 81(3): 543-550.
[14] Jamieson, D., (1998): Oxidative stress responses of the yeast *Saccharomyces cerevisiae*. Yeast, 14(16): 1511-1527.
[15] Kostovic, K., Pastar, Z., Pasic, A., Ceovic, R., (2007): Treatment of vitiligo with narrow-band UVB and topical gel containing catalase and superoxide dismutase. Acta Dermatovenerologica Croatica, 15(1): 10-14.
[16] Laitaoja, M., Valjakka, J., Janis, J., (2013): Zinc Coordination Spheres in Protein Structures. Inorganic Chemistry, 52(19): 10983-10991.
[17] Leonard, S., Gannett, P., Rojanasakul, Y., Schwegler-Berry, D., Castranova, V., Vallyathan, V., Shi, X., (1998): Cobalt-mediated generation of reactive oxygen species and its possible mechanism. Journal of Inorganic Biochemistry, 70(3-4): 239-244.
[18] Lowry, O., Rosebrough, N., Farr, A., Randall, R., (1951): Protein measurement with the folin phenol reagent. The Journal of Biological Chemistry, 193(1): 265-275.
[19] Marnett, L., (2000): Oxyradicals and DNA damage. Carcinogenesis, 21(3): 361-370.
[20] Mates, J., Perez Gomez, C., Blanca, M., (2000): Chemical and biological activity of free radical scavengers in allergic diseases. Clinica Chimica Acta, 296(1-2): 1-15.
[21] Morano, K., Grant, C., Moye-Rowley, W.S., (2012): The Response to Heat Shock and Oxidative Stress in *Saccharomyces cerevisiae*. Genetics, 190(4): 1157-1195.
[21] Muthukumar, K., Nachiappan, V., (2010): Cadmium-induced oxidative stress in *Saccharomyces cerevisiae*. Indian Journal Biochemistry Biophysics, 47(6): 383-387.
[23] Sravani, P., Babu, N., Gopal, K., Rao, G., Rao, A., Moorthy, B., Rao, T., (2009): Determination of oxidative stress in vitiligo by
measuring superoxide dismutase and catalase levels in vitiliginous and non-vitiliginous skin. Indian Journal Dermatology Venereology Leprology, 75(3): 268-271.
[24] Turrens, J., (2010): Superoxide dismutases and catalases. Comprehensive Toxicology (Charlene McQueen, Ed.), Oxford: Elsevier, 4: 219-227.
[25] Usatîi, A., Chiselita, O., Rudic, V., Chiselita, N., Molodoi, E., Efremova, N., Gulea, A., Turta, C., Lozan, V., (2012): The effects of some compounds of Mn(II) and Zn(II) on the multiplication of wine yeast and biosynthesis of carbohydrates in the biomass**.** Analele Universităţii din Oradea, Fascicula Biologie, 17(2): 306-312.
[26] Valko, M., Morris, H., Cronin, M., (2005): Metals, toxicity and oxidative stress. Current Medicinal Chemistry, 12(10): 1161-1208.

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