REGULATION OF BIOSYNTHESIS PROCESS OF CELLULAR COMPONENTS IN *Saccharomyces cerevisiae* WITH <50 nm ZnO NANOPARTICLES APLICATION

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Abstract. The paper provides new data about the influence of ZnO nanoparticles with <50 nm dimensions in concentrations 1, 5, 10, 15, 20, 30, 50, 70 mg/L on cell viability, biomass production, total carbohydrates, proteins, β-glucans and catalase activity of *Saccharomyces cerevisiae* CNMN-Y-20 yeast strain. Our research has demonstrated the efficiency of ZnO nanopaticles utilization for stimulation of biosynthetic processes at studied strain. Nanoparticles stimulate cell viability, biomass production, protein content, β-glucans and insignificantly carbohydrates content, their effect depends from used concentration. A statistically significant increase was found in β-glucans content on using 10 and 15 mg/L concentrations of nanoparticles. The obtained results highlights the perspective of using <50 nm ZnO nanoparticles in yeast biotechnology for obtaining of β-glucans.

Keywords: *Saccharomyces cerevisiae,* ZnO nanoparticles, multiplication, biomass, cellular components.

INTRODUCTION

Applying of nanoparticles at microorganisms cultivation is an innovative field in nanobiotechnology research [19, 21]. One of the dangers, recognized today by most researchers, is the insufficiency of information on the influence of nanomaterials on living organisms, including humans. Thus, it is important to determine the conditions when to use safe nanotechnologies for living systems, especially for humans, a problem that is frequently discussed in numerous scientific papers [26].

Currently ZnO is considered one of the richest materials amongst the family of nanostructures of metal oxides [29]. ZnO is comparatively inexpensive, biocompatible and relatively less toxic compared with other metal oxide nanoparticles, which further supports its application potential [24]. It is bio-safe, with distinctive abilities such as structure-dependent, electrical and thermal transport properties, that might vary according to the particle size, shape, morphology, orientation and ratio [18]. Among different nanomaterials, ZnO nanoparticles possess a special status due to their high specific surface area, optical transparency, chemical and photochemical stability, convenient fabrication, high-electron communication features and electrochemical activities [23].

In the papers of many authors the results of the influence of different nanoparticles (oxides of Au, Ag, Ti, Si, Zn) on the microbial cell are exposed, from which we conclude that, depending on their structure and concentration, they can stimulate or inhibit growth and biosynthetic activity of microorganisms [10].

The metabolic complexity of microorganisms complicates the analysis and identification of the nanoparticle-cell interaction. In order to better understand the mechanisms of influence of nanoparticles on microorganisms, it is important to highlight the effects of nanoparticles on different microbial population development indicators, including biosynthesis processes of cellular components. A complete understanding of how cells interact with different nanostructures remains little studied. Possible mechanisms of nanoparticles influence at the cellular level have been investigated by several specialists in the field, which have elucidated some processes that occur when applying nanoparticles [11, 13, 17].

In general, changes in the cell's physiological state and reaction to external factors influence the structure and dynamics of the cell wall. According to Ya-Nan Chang research [6], the mechanism of influence of CuO and ZnO nanoparticles on the microbial cell is complex and attracts changes in both the cell membrane and the cytoplasm. A study evaluating the influence of TiO_2, ZrO_2 and Fe_2O_3 nanoparticles on O_2 consumption and cell membrane integrity in *S. cerevisiae* yeast performed Lila Otero-Gonzalez, which established that concentrations up to 1000 mg/L are not toxic to yeast [20].

From the above mentioned, the aim of the research was to evaluate the effects of ZnO nanoparticles with <50 nm dimensions on the development of *Saccharomyces cerevisiae* CNMN-Y-20 yeast strain and its biosynthetic potential.

MATERIALS AND METHODS

Research object. As an object of research served *S. cerevisiae* CNMN-Y-20 strain, producer of β-glucans, preserved in the National Collection of Nonpathogenic Microorganisms of Institute of Microbiology and Biotechnology [7].

Media and culture conditions. For inoculation and submerged yeast cultivation were used the fermentation media YPD specific for studied strain (g/L: yeast extract -10.0, peptone - 20.0, glucose-20.0) [2] and beer wort [4]. Submerged cultivation was carried out in 1.0 L Erlenmeyer flasks on a stirrer with a rotating speed of 200 r.p.m., at a temperature of 25°C, an aeration degree of 81.3...83.3 mg/L, the duration of submerged cultivation - 120 hours. The liquid fermentation medium was innoculated in 5% volume with the inoculum of $2x10^6$ cells/mL.

Nanomaterials. ZnO nanoparticles with dimensions <50 nm in form of nanopowder, purity >97%, contains 6% Al dopant, surface area >10,8 m^2/g (ALDRICH) were used. The suspension of nanoparticles was prepared according to the method specified [20]. The concentrations of nanoparticles used in experiments constituted 1; 5; 10; 15; 20; 30; 50 and 70 mg/L. Nanoparticles were added at the time of inoculation with the seed material. The variant without application of nanoparticles was used as control sample.

Methods of achieving research. *Cell viability* was determined by counting the number of colonies formed on the Petri dishes after 6 and 24 hours of cultivation using method of strain exhaustion. For yeast cultivation was used beer wort solid medium. Incubation was carried out at 28°C for 5 days. Calculation formula CFU/mL = number of colonies x dilution factor x 10 [22, 31]. *Cell biomass* was determined gravimetrically [13]. *Proteins* was determined by the Lowry method [16], using crystalline albumin from bovine serum as standard. *Total carbohydrates* in the yeast biomass were determined at the spectrophotometer PG T60 VIS Spectrophotometer at wavelength 620 nm using Anthrone reagent and D-glucose as standard [8]. The *β-glucans* content in the yeast biomass was determined gravimetrically as described [27]. *Catalase activity* was determined by the methods [1, 9]. Statistical analysis of results was done using statistical software kit 7, veracity compared to the control - $p \leq 0.05$.

RESULTS

During research, in order to obtain ample information on the effects of nanoparticles on yeast growth and metabolism, in parallel with the determination of β-glucans content in cell wall, the viability of modified cells was evaluated, established biomass production, carbohydrate and protein contents in yeast biomass, determined catalase activity.

The cell viability is an important criterion in appreciation of the negative or positive effects of exogenous factors. Investigations have shown that ZnO nanoparticles stimulate cell viability of *S. cerevisiae* CNMN-Y-20 strain after 6 hours and after 24 hours of cultivation in concentrations of 1-50 mg/L, it was found that the viability rate is in evident increase compared to the control. Only the concentration of 70 mg/L reduces the cell viability which is under the control sample level (Figure 1). The maximal stimulating effect on cell viability after 6 hours of cultivation is observed at application of 5 mg/L and constituted 104% more than the control sample and after 24 hours maximum stimulatory effect on cell viability (by 136%) is observed upon application of 20 mg/L nanoparticles. We can conclude that the stimulatory effect depends on the concentration of nanoparticles.

The evaluation of the ZnO nanoparticles effect on biomass production, protein and carbohydrate contents, β-glucans, activity of the antioxidant enzyme - catalase in *S. cerevisiae* CNMN-Y-20 yeast was performed after 120 hours of submerged cultivation.

In the process of monitoring the effects of nano-oxide on the accumulation of cell biomass at 1 L of culture medium, significant differences in biomass production values were established. The amount of dry biomass in the control sample is 4.82±0.59 g/L, in the experimental variants its production varies around the 5.06-5.8 g per 1 L culture medium, which is with 5-20.3% more than the control (Figure 2).

The positive influence of nanoparticles on protein biosynthesis processes is evident. Investigations indicates that nanoparticles used in the culture medium at concentrations of 5 to 70 mg/L increase protein content by 10…23.4% compared the control. The maximum amount of proteins in yeast biomass (50.5±6.15%) was observed on the cultivation in the presence of 70 mg/L nanoparticles. In the biomass of the control sample the proteins are 40.99±4.54% (Figure 2).

The results analysis of the carbohydrate content determination in yeast biomass indicates on relative stability for variants where nanoparticles were applied at concentrations of 1-70 mg/L. From the results presented in Figure 2, the maximum amount of carbohydrates was recorded under the influence of ZnO nanoparticles at the concentration of 30 mg/L and constituted 16.6% more than the control sample.

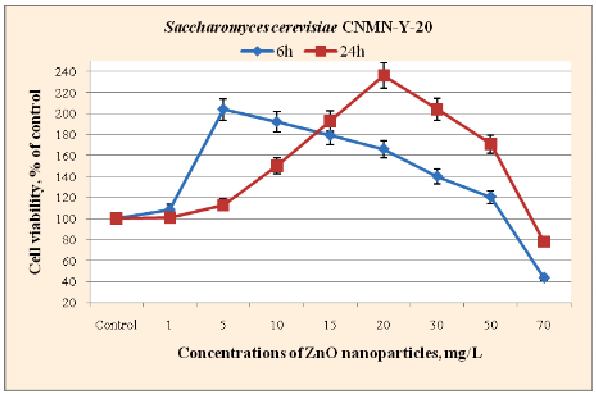

Figure 1. Cell viability of *S. cerevisiae* CNMN-Y-20 under the influence of ZnO nanoparticles

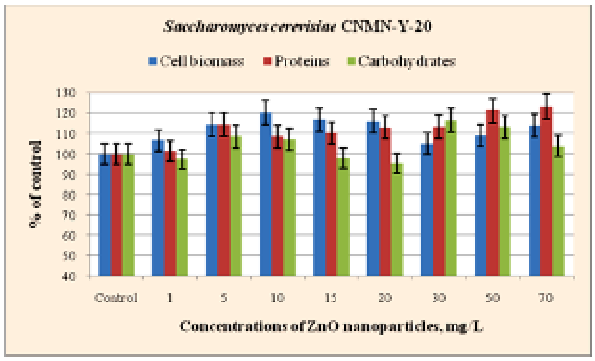

Figure 2. Biomass production, protein and carbohydrate contents in *S. cerevisiae* CNMN-Y-20 under the influence of ZnO nanoparticles

Research on quantitative determination of β-glucans in yeast biomass at cultivation in the presence of ZnO nanoparticles (<50 nm) revealed positive modifications. The β-glucans content in dry biomass in the experimental samples varied from 19.91% to 23.73%, in the control sample β-glucans constituted 17.82±1.06%. A statistically significant increase (with 30.3-33.2%) in β-glucans content was found for concentrations of 10 and 15 mg/L (Figure 3). Because the research has used nanoparticles that are little studied in the biotechnology of β-glucans, producing by yeasts, the issue of polysaccharide production is still in the focus of attention. In this regard, a study to elucidate the maximum effective concentration to obtaine increased β-glucans production was initiated. Generalizing the experimental results of β-glucans production, calculated on 1 L culture medium, we can affirm that the maximum product amount is obtained using 10-15 mg/L of ZnO nanoparticles. These concentrations ensure 1.335-1.346 g/L β-glucans, which is with 54.9-56.1% more than the control (Figure 3).

One of the significant tests for nanoparticle action is the activity of the antioxidant enzyme - catalase. It can be seen from Figure 4 that the response of the yeast strain to the introduction of nanoparticles at concentrations of 1-10 mg/L is expressed by increasing catalase activity by up to 35%. Then, with increasing of concentration, we observe a gradual decrease in enzyme activity and an inhibition of catalase on using of maximum concentrations (50, 70 mg/L). These fluctuations in the catalase activity are explained by the

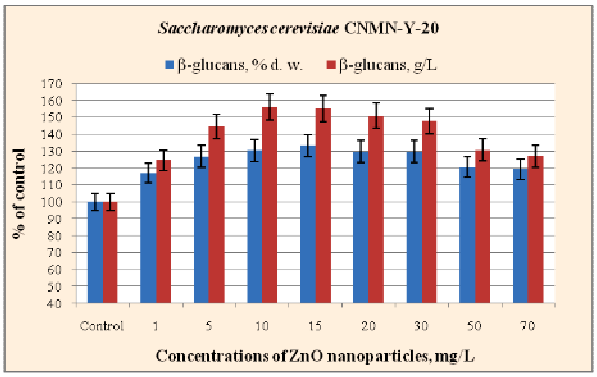

Figure 3. β-glucan content in *S. cerevisiae* CNMN-Y-20 under the influence of ZnO nanoparticles

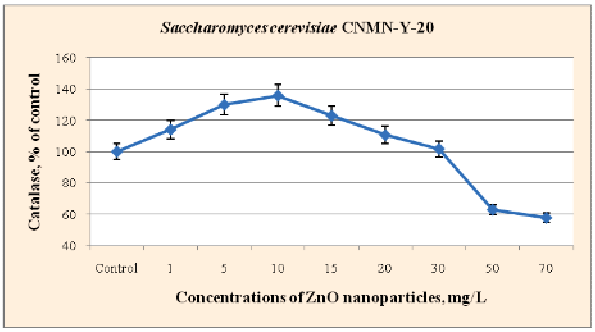

Figure 4. Catalase activity of *S. cerevisiae* CNMN-Y-20 under the influence of ZnO nanoparticles

fact that natural antioxidant components are the firsts which react to environmental toxic factors having the function of stabilizing and neutralizing the negative effects on the cell.

The analysis of experimental data demonstrates that <50 nm ZnO nanoparticles lead to stimulation of β-glucans, biomass production, protein and carbohydrate values of the *S. cerevisiae* CNMN-Y-20 strain at concentrations ranging from 1 to 70 mg/L. Concentrations of 10-15 mg/L are proposed as an inducer of biosynthesis processes to improve the β-glucans production. The results allow to propose a new process in the biotechnology of *S. cerevisiae* CNMN-Y-20 yeast cultivation.

DISCUSSION

This paper presents the synthesis of the results obtained in studying the influence of ZnO nanoparticles with dimensions <50 nm on the viability, biomass productivity, biosynthetic activity and catalase activity of *S. cerevisiae* CNMN-Y-20 yeast - producer of β-glucans.

Several researchers report on selective toxicity and antimicrobial activity of ZnO nanoparticles [14, 30]. The toxicity degree of the nanoparticles depends on several factors: concentration, dimension and used method of their synthesis. Thus, it is important to determine the nanoparticle concentrations, favorable for the productivity and biosynthetic activity of the studied strain.

Tests for cell viability determination by counting colony forming units (CFU) are most often used to determine the toxicity or inoffensiveness of different types of chemicals, nanocomposites or biomaterial compounds [22]. In our case, the obtained results on the study of cell viability of yeasts demonstrate that for both 6 hours and 24 hours of strain cultivation, it is in evident increase, but use of 70 mg/L concentration the viability decreases to 43.6 and 78.4% respectively, indicating that the concentration of 70 mg/L is toxic to the studied strain. These results are similar to the results obtained by other researchers. For example, Garcia-Saucedo et. al. mention that nanoparticles ZnO in concentrations more than 50 ppm (50 mg/L) were characterized by toxic effect for *S. cerevisiae* [12].

It is known from the specialty literature that zinc is a microelement strictly necessary for the structure, multiplication, growth and development of yeasts, consisting of about 300 enzymes that mediate metabolic processes, DNA and RNA synthesis and proteins [25].

The study of the influence of nanoparticles on biomass production showed that its values are higher compared to the control sample in all experimental variants. The maximum effect was observed on using of the 10 mg/L concentration at which the biomass amount was 5.8±0.4 g/L and constituted 20.3% more than the control.

For us, it was necessary to determine how zinc oxide nanoparticles work on the accumulation of proteins and total carbohydrates, which are the main sources of energy for the cell. From the results we can conclude that nanoparticles act positively on these indicators. Carbohydrate content is maximal on using 30 mg/L nanoparticles. The influence of other concentrations is insignificant. In the study of the action of nanoparticles on the protein content a more pronounced positive influence is observed. Our research data demonstrate the stimulation of proteins at concentrations ranging from 5 to 70 mg/L, maximum being for 70 mg/L concentration and is by 23.4% more than the control sample.

The significant increase in catalase activity in the studied strain was observed on using the 10 mg/L concentration, the enzyme level was 35% higher than in the control variant. Data from the literature demonstrate the possibility of using ZnO nanoparticles to stimulate enzyme synthesis. Thus, Ban et al. report about perspective on the potential of ZnO nanoparticles utilization as supplement in order to increase active production of β-glucosidase - enzyme of *S. cerevisiae* with industrial value, as well as to increase production of baker's yeast [5]. Concentrations of 50-70 mg/L nanoparticles lead to decreased catalase activity, indicating their toxicity for the yeast strain.

Further, our studies have focused on evaluation of the nanoparticles effect on β-glucans content - one of the main components of the yeast cell wall. The high interest on β-glucans is conditioned by their antibacterial, antitumor, antioxidant, antimutagenic, hypocholesterolemic, detoxifying activities, etc. [3, 15, 28]. The experimental results have shown that nanoparticles increase β-glucans content in the studied strain at all concentrations. The maximum of β-glucans were observed at 10-15 mg/L at which the β-glucans content were 23,22±0,55-23,73±0,32% d. w. and constituted 30.3-33.2% more than the control. Their amount, calculated on 1 L of culture medium, allows obtaining 1,335-1,346 g/L β-glucans, which is 54.9-56.1% more than the control. The obtained results led to the elaboration of a new process of yeast cultivation and stimulation of β-glucans content.

Thus, we can affirm that ZnO nanoparticles cause modifications in yeast metabolism, stimulating their biosynthetic potential and the effect depends on the used concentration. Our results demonstrate the absence of toxicity of ZnO nanoparticles (<50 nm) applied in the YPD culture medium at concentrations of 1 to 50 mg/L and the possibility of their utilization in the *S. cerevisiae* CNMN-Y-20 yeast cultivation biotechnology.

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