REACTION OF *Saccharomyces cerevisiae* YEAST STRAIN TO THE ACTION OF TiO_2 NANOPARTICLES

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Abstract. The paper provides new data about the effect of TiO_2 nanoparticles with dimensions of 40 nm on bioproduction parameters of *Saccharomyces cerevisiae* CNMN-Y-18 strain. According to the obtained results, TiO_2 nanoparticles added to the YPD medium in concentrations of 10, 20, 30, 40 mg/L did not seriously affect the growth of the yeast strain. The values of cellular biomass production, of total carbohydrate, mannoprotein and protein content, catalase activity fit in the range of the admissible error compared with the control. Monitoring of the evolution of biosynthetic processes in the yeast biomass in correlation with the concentration of nanoparticles is of great interest for the technologies of yeast biomass final product obtaining.

Keywords: *Saccharomyces cerevisiae;* nanoparticles; biomass; carbohydrates; mannoproteins; proteins; catalase.

INTRODUCTION

Certain types of nanoparticles are currently studied for further application in medicine (early identification of diseases), cosmetics (sun-screen protection lotions, tooth pastes), food packaging, food colorings, textile industry etc. [2, 18, 27, 30]. Titanium dioxide (TiO_2) nanoparticles (NPs) are obtained for a wide spectrum of applications. Recent studies have highlighted that TiO_2 nanoparticles are highly stable, envirnonment-friendly and bioactive, in comparison with other agents. The data on biotechnological application of TiO_2 nanoparticles in different fields are widely reviewed [6, 10, 21]. A major problem of nanoparticles use consists in the evaluation of the potential risks for the environment and human health. Currently, the utilizatin of nanotechnologies is controlled by the European Community regulation regarding the chemicals and their use in secure conditions – Registration, Evaluation, Authorization and Restriction of Chemical substances **(**REACH) [11, 25]. In this context the investigation on identification of inorganic nanoparticles effects on the environmental objects are actual. Regarding the level of toxicity of TiO_2 nanoparticles on the growth of yeasts from genus *Saccharomyces,* Kasemets [15] mentions that nanoparticles with concentration of 20 000 mg/L are not toxic.

Besides their wide use as biotechnological producers, eukaryotes are of great interest for nanotechnologies as model organisms for studies of biocompatibility or biotoxicity of different nanoparticles and nanomaterials synthesized by abiotic procedures. Yeasts are representative objects that offer enormous possibilities in modeling the effects and identification of the action mechanisms of different factors on cell's vital processes. These characteristics make yeasts reliable models for identification of the effects of different nanoparticles. Biological and biochemical indicators are proposed as landmarks.

An innovative aspect of nanoparticles application refers to the elucidation of the possibilities of increase of biotechnological performances of microorganisms producing of biologically active substances.

The aim of the current study is to highlight the reaction of *Saccharomyces cerevisiae* CNMN-Y-18 yeast strain at the action of TiO_2 nanoparticles in order to model the biosynthetic processes of some bioactive principles.

MATERIALS AND METHODS

Object of study. For this study we used *Saccharomyces cerevisiae* CNMN-Y-18 yeast strain that is a producer of mannoproteins [29]. The strain is preserved in the collection of Yeasts Biotechnology Laboratory and in the Collection of Nonpathogenic Microorganisms of the Institute of Microbiology and Biotechnology of the Academy of Sciences of Moldova.

Culture Media. For inoculation and submerged cultivation of yeasts was used fermentation media specific to strains in YPD (10 g l^{-1} yeast extract, 20 g l^{-1} bactopeptone and 20 g l^{-1} glucose) study [3]. Submerged cultivation was carried out in Erlenmeyer flasks with a capacity of 1.0 L, the rotating speed of the stirrer 200 rpm, at 25^0C, the degree of aeration 80.0...83.0 mg/L, the length of submerged cultivation 120 hours. Broth medium was seeded in an amount of 5% with the inoculum 2 x 10^6 cells/mL. The oxygen content was measured with the portable oximeter – Oxi-315i/SET 2B10-0011. The pH values of the cultivation environment were measured with the pH-316i MeB ketten WTW, Germany.

Nanomaterials. For the purposes of the current study we used TiO_2 nanoparticles of 40 nm, stabilized in polyvinylpyrrolide (PVP), which were kindly offered by the researchers of the Institute of Electronic Engineering and Nanotechnologies ,,D.Ghitu" of the Academy of Sciences of Moldova. Nanoparticles concentrations used for yeast cultivation were of 10, 20, 30, and 40 mg/L. In the control sample there were used no nanoparticles.

Methods of achieving research. Yeast biomass was determined gravimetrically [13]. Total carbohydrates in

the biomass of yeast were determined with PG T60 VIS Spectrophotometer at wavelength 620 nm using Anthrone reagent and D-glucose as standard [7]. Protein was determined spectrophotometrically, according to the method of Lowry [17], using crystalline albumin from bovine serum as standard. Mannoprotein content was determined gravimetrically, according to the protocols: 10 g dry weight of yeast were first sieved, then the yeast suspension was centrifugated at 4500 g for 10 min, followed by re-suspension of the deposition in 50 ml sterile distilled water. The procedure was repeated 5–6 times until the supernatant was clear, then the yeast was purified. After purifying, 0.5 L of 2% NaOH (w/v) was added to the cell wall sediment. This was placed in a boiling water bath and agitated at 150 rpm/min for 2 h. The preparation was centrifuged and the supernatant was collected. The residue was washed with little deionized water and combined with supernatant extracts. After that, the pH was adjusted to 6.5 with 10% acetic acid, and the supernatant was concentrated to one fifth of the original volume, triple absolute ethyl alcohol were added to precipitate mannan. The precipitated mannoprotein was dissolved in water and centrifuged, the supernatant was precipitated again by the addition of triple ethanol and recentrifuged. The resultant white sediment was washed twice with absolute ethanol and once with ether, then dried at 70 ^{0}C. [14]. Catalase activity was determined by the described methods [1, 9]. Statistical processing of the obtained results was made electronically with the calculation of the standard errors for the relative and average values; the differences between the experimental and control data were established using Student's t-test and P value.

RESULTS

The utilization of *Saccharomyces cerevisiae* CNMN-Y-18 yeast strain as a model organism is determined by the various content of bioactive principles in the biomass and an advanced level of adaptation to the action of the external factors. The production of cellular biomass, the content of total carbohydrates, mannoproteins, proteins and catalase activity in the biomass were tested as indicators of modifications in yeasts that were subjected to the action of nanoparticles.

In our study we determined that TiO_2 nanoparticles in concentrations of 10 – 40 mg per 1 L of culturing medium do not show any activity on the biomass production process (Fig. 1a). Together with the increasing of the nanoparticles concentration in the culturing medium we observed a non-semnificative tendency of decrease of cellular biomass, values that were at the limit of the admissible error. The regression analysis that has a predictive value and represents the mathematical relationship of the dependency, showed that its ecuation was y = -0.187x + 101.8. The correlation ratio confirms an average dependency of the productivity values of the studied yeast with the nanoparticles concentrations, the coefficient of determination being moderate ($R^2 = 0.833$) (Fig. 1b).

Further, we determined the content of total carbohydrates and the polysaccharide component – mannoproteins – in the yeast biomass. We observed that in the presence of TiO_2 nanoparticles the content of carbohydrates increased in all the experimental variants, the increase being of 10.5-13.5% in comparison with the control (Fig. 2a). The correlation

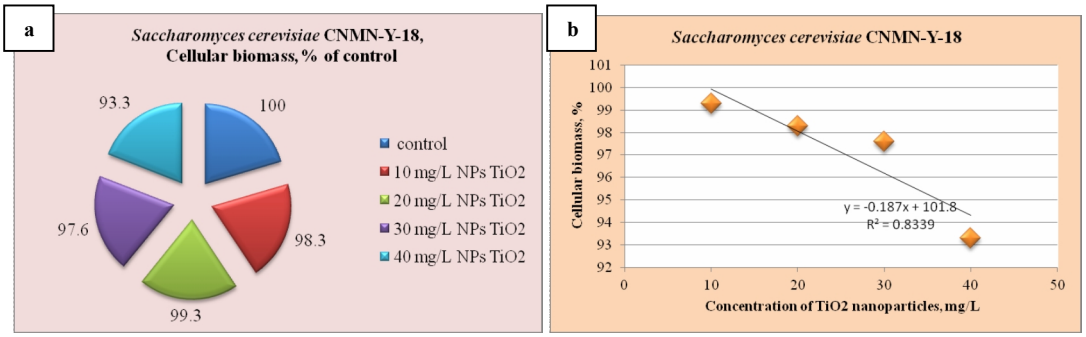

Figure 1. *Saccharomyces cerevisiae* CNMN-Y-18 biomass production during cultivation in the presence of TiO_2 (40 nm) nanoparticles

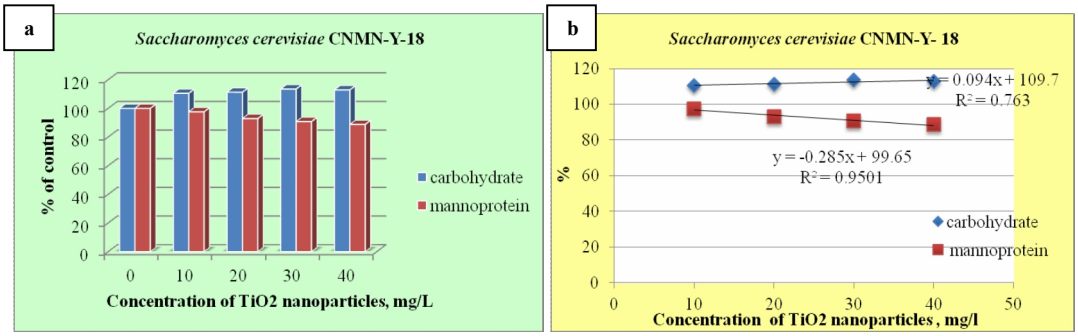

Figure 2. Carbohydrate and mannoprotein content in *Saccharomyces cerevisiae* CNMN-Y-18 biomass during cultivation in the presence of TiO_2 (40 nm) nanoparticles

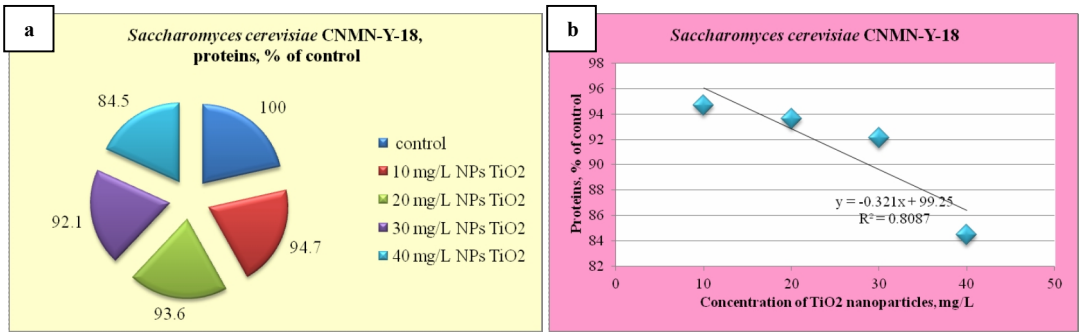

Figure 3. Protein content in *Saccharomyces cerevisiae* CNMN-Y-18 biomass during cultivation in the presence of TiO_2 (40 nm) nanoparticles

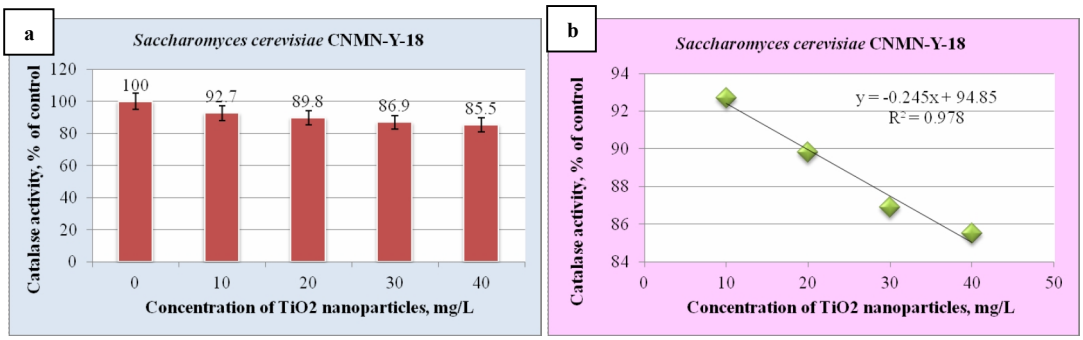

Figure 4. Catalase activity of *Saccharomyces cerevisiae* CNMN-Y-18 biomass during cultivation in the presence of TiO_2 (40 nm) nanoparticles

ratio confirms the dependency of the values of carbohydrate content in yeast biomass with those of nanoparticles concentrations, the determination coefficient being ($R^2 = 0.763$) (Fig. 2b).

The explorative analysis of the obtained data regarding the mannoprotein content in *Saccharomyces cerevisiae* CNMN-Y-18 yeast strain during cultivation in the presence TiO_2 nanoparticles with different concentrations, showed a slight decrease, which strongly correlates with the nanoparticles concentrations. The coefficient of determination in this case $R^2 = 0.950$ (Fig. 2b).

We observed similar effects of the TiO_2 nanoparticles when we determined the protein content in the yeast biomass. In the experimental samples where we added nanoparticles the protein content ranged 24.4 – 27.32 % of the dried substance, in comparison with the control where the protein content consisted 28.85% of the dried substance. The ratio of the protein content values from the experimental samples and the control samples varied 94.7% for nanoparticles with concentration 10 mg/L and 84.5% in the case of nanoparticles with concentration 40 mg/L (Fig. 3a). The correlation ratio indicates that TiO_2 nanoparticles concentrations greatly influence the protein content, the determination coefficient being $R^2 = 0.808$ (Fig. 3b).

Regarding toxicity of a great importance are the values of antioxidant enzymes. The cumulative results of catalase activity in the samples with different concentrations of TiO_2 nanoparticles correspond with our data on the determination of the effects of nanoparticles on the biochemical components from the yeast biomass. The catalase activity slightly decreased non-significantly at the increase of nanoparticles concentrations (Fig. 4a). In absolute values, at the maximum nanoparticles concentration of 40 mg/L we observed a decrease of catalase values by 13.1-14.5% in comparison with the control sample, which indicates that the yeast cell is not able to effectively neutralize the toxic compound. The determination coefficient $R^2 = 0.97$ confirms a dependency between the values of catalase activity and the values of nanoparticles concentration (Fig. 4b).

The general conclusion of the current study is that the bioproductive parameters of *Saccharomyces cerevisiae* CNMN-Y-18 yeast strain, during cultivation in the presence of TiO_2 nanoparticles almost do not change, except the content of total carbohydrates, whose amount increased by 13.5% in comparison with the control sample. In the studied experimental conditions, the catalase plays an antioxidant role in the nano-modified yeast biomass.

DISCUSSION

This paper presents the results of a research on the influence of the TiO_2 nanoparticles of 40 nm on *Saccharomyces cerevisiae* CNMN-Y-18 yeast. The functionality of TiO_2 nanoparticles is mentioned in different publications that confirm the efficiency of their use in food industry, medicine, in dye and pigment production, cosmetics. The application of inorganic nanoparticles at cultivation of microorganisms represents a recent field in nanobiotechnological research [8, 22, 23]. Some data hypothesize that many nano-composites manifest toxic activity, as well as stimulating action over the metabolic processes in microorganisms, which

manifest depending on the applied concentration [19, 24, 26]. This study demonstrated the high toxicity of Mn_2O_3 and the low or no toxicity of Fe_0, Fe_2O_3, TiO_2, and ZrO_2 NPs to the eukaryotic cell model microorganism *S. cerevisiae* yeast [19].

Our results regarding the determination of TiO_2 influence on the biomass production of *Saccharomyces cerevisiae* CNMN-Y-18 indicate at a non-significant tendency of its decrease at the increase of nanoparticles concentration from 10 to 40 mg/L cultivation medium (Fig. 1). The yeasts from *Saccharomyces* genus are frequently used as technological objects to obtain glucidic preparations. The main groups of polysaccharides that can be obtained from yeast are β-glucans and mannoproteins. Due to their specific properties, mannoproteins can be used as preparations with immunomodulating, antioxidant and anti-mutagen effects [14, 16]. Another direction is mannoprotein application in food industry. Mannoproteins are used as thickening, stabilizing and dispersing agents, as gelling substances in syrup and jam production, replacing those of vegetal origin [5]. Mannoproteins obtained from yeast are of great interest for oenology. Due to their specific interaction with anthocyans and tannins, mannoproteins directly contribute to the stabilization of color and reduction of astringency of wine [12]. These data convince us about the necessity to study the use of NPs in the cultivation technology of yeasts as a strategy of boosting the biotechnological performancies of commercial mannoprotein production.

Our experimental result with *Saccharomyces cerevisiae* CNMN-Y-18 strain showed that in the presence of 40 nm TiO_2 nanoparticles the content of total carbohydrates increased in all the experimental samples. At the same time the content of mannoproteins slightly decreased in yeast biomass during cultivation with nanoparticles with different concentrations, which strongly correlates with the nanoparticles concentration. The determination coefficient is a very string one $R^2 = 0.950$ (Fig. 2). Previous research on evaluation of 30 nm TiO_2 nanoparticles effect, applied in smaller concentrations (0.5-15 mg/L), determined a significant boost in mannoprotein accumulation ratio in *Saccharomyces cerevisiae* CNMN-Y-18 strain (by 19-22.6%) [28].

According to many studies, in terms of toxicity, of a great importance are the values of activity of antioxidant enzymes [4, 20]. Catalase is particularly important for the life of organisms, playing roles in cellular respiration and defense against oxidative stress. In our experiments where we applied 40 nm TiO_2 nanoparticles in different concentrations, the cumulative results of catalase activity showed a slow decrease linked with the increase of the nanoparticles concentrations used for *Saccharomyces cerevisiae* CNMN-Y-18 yeast strain (Fig. 4).

The general conclusion is that 40 nm TiO_2 nanoparticles in concentrations of 10, 20, 30, 40 mg/L, added to the YPD culture medium, did not significative alter the development of *Saccharomyces cerevisiae* CNMN-Y-18 strain. The values of cellular biomass production, of total carbohydrate, mannoprotein, protein content, catalaze activity range in the limit of the allowable error in comparison with the control sample. In summary, this study demonstrated the low or no toxicity of TiO2 nanoparticles to yeast cells. Monitoring of the evolution of biosynthetic processes in the yeast biomass in correlation with the concentration of nanoparticles is of great interest for the technologies of yeast biomass final product obtaining.

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