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EFFECT OF Fe_3O_4 AND TiO_2 NANOPARTICLES ON CATALASE ACTIVITY AND β-CAROTENE CONTENT AT PIGMENTED YEAST STRAIN *RHODOTORULA GRACILIS*

– Research paper –

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Abstract: The properties of nanoparticles have been used in a wide range of potential applications in food industry, medicine, microbial biotechnology, cosmetics, environmental production. Research results of evaluation of the effect of Fe_3O_4 and TiO_2 nanoparticles applicated in large concentration limits on some parameters of pigmented yeast strain *Rhodotorula gracilis* CNMN-Y-30 are presented in this paper. It was established that nanoparticles selected for study caused toxic effects on β-caroten accumulation and activity of antioxidant enzyme catalase depending on concentration and nanostructure. The strong correlation between concentration of nanoparticles and evaluated components has been revealed at studied yeast strain. Coefficients of correlation varied in limits R^2 = 0.623... 0.951. For the first time, the determination of β-carotene accumulation and catalase activity rate as functional tests for estimation of toxicity of nanoparticles for pigmented yeast strain *Rhodotorula gracilis* was effectuated. Thus, it was established that adaptive response of yeast strain to the presence of Fe_3O_4 and TiO_2 nanoparticles has been manifested by modification of the processes of β-carotene biosythesis and catalase activity.

Keywords: *yeast,* nanoparticles *Fe_3O_4, TiO_2, Rhodotorula gracilis*, β-carotene, catalase.

INTRODUCTION

New fundamental aspects of aplicability of nanoparticles, including the synthesis of materials at nanometric scale, the utilization of physicochemical and optoelectronical properties have been revealed due to intensive development of nanotechnology (Murray et al., 2008). Experimental results obtained previously have demonstrated that nanoparticles (NPs) have wide range of applications in areas such as environmental protection, health care, food industry, cosmetology and pharmaceutical industry (Mohapatra et al., 2010; Rai et al., 2011; Buteică, 2014).

An important field for nanoparticles utilization represents food industry. Nanomaterials possess the capacity to improve the nutritional quality of foods, to increase the validity of foods being efficient in the preservation. Also, some nanoparticles have antimicrobial activity and could be used in the process of food packaging and serve as packaging material (Sastry et al., 2011; Chen et al., 2014). Until the present, organoleptic properties of food products such as candies, chewing gum, oils, food flavors were improved because of nanoparticles utilization (Weir et al., 2012). The implication of nanomaterials in food industry and technology could satisfy population demand for superior quality food products. The utilization of metal oxide nanoparticles such as Fe_3O_4, TiO_2, Ag with significant potential, simple preparation and high stability is of great interest for researchers (Yang et al., 2010; Sparrenberger et al., 2015). In that regard, data for toxicity of nanoparticles and their effect on living organisms and cellular components are needed for the application of nanoparticles in health care and environmental protection. The utilization of microorganisms, especially yeasts, as the object of study for the analysis of the toxicity of nanoparticle, has a lot of advantages being representative subjects for regulation of vital processes and establishment of mechanisms of nanoparticles action on cellular components (Beşliu et al., 2016). In microbial biotechnology, for functional test of

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measurement of inhibition or stimulation rate of any compound that is manifested by 50% of maximum effect (dose/answer), the utilization of such terms as efficient concentration ($EC_{50\%}$) or inhibitory concentration ($IC_{50\%}$) of compound (Sebaugh, 2011) is proposed. These tests offer the possibility to realize the complete study of the toxicity of nanoparticles. In that context would therefore be important the research destined to the determination of the toxicity grade of nanoparticles on yeasts with the aim to elaborate a new cellular model and to establish the toxicity of nanoparticle for further valorification of its potential.

Thus, the aim of this research was to determine the effects of nanoparticles Fe_3O_4 (10 and 30 nm) and TiO_2 (30 nm) on catalase activity and β-carotene content at the cultivation of pigmented yeast strain *Rhodotorula gracilis* CNMN-Y-30. The obtained results can be used for the further development and validation of tests for nanotoxicology studies in vivo.

MATERIALS AND METHODS

Objects of study. Pigmented yeast *Rhodotorula gracilis* CNMN-Y-30 was selected for the study of the effect of selected nanoparticles on yeasts. The strain is preserved in the collection of Yeasts Biotechnology Laboratory and in the Collection of Nonpathogenic Microorganisms of Institute of Microbiology and Biotechnology of Academy of Sciences of Moldova. *Nanomaterials***.** In the experiments Fe_3O_4 nanoparticles (10 and 30 nm) and TiO_2 (30 nm) were used, the suspension was prepared according to the method specified (Oterro-Gonzalez et al., 2013) made available for us with great kindness by researchers of the Institute of Electronic Engineering and Nanotechnologies "D. Ghitu" of the Academy of Science of Moldova. The dimensions of Fe_3O_4 nanoparticles were selected according to the previously obtained results of the influence of these nanoparticles with dimension of 10 and 30 nm on cells proliferation, production of biomass, carbohydrate and protein content (Usatii et al., 2016). The dimension of TiO_2 (30 nm) nanoparticles was selected according to the research under development. The concentrations of nanoparticles used in experiments to cultivate yeasts constituted 0.5; 1.0; 5.0; 10 and 15 mg/L. The sonicated suspension of nanoparticles in volume that corresponds to studied concentration was added to the nutritive medium simultaneously with inoculum. The variant without application of nanoparticles was used as control sample.

Culture media. For inoculation and submerged cultivation of yeasts was used fermentation media specific to strains in YPD study and beer wort (Aguilar-Uscanga et al., 2003). The submerged cultivation was carried out in depth capacity 1 liter Erlenmeyer flasks on shaker (200 rpm.) at a temperature of +25ºC, aeration rate 80,0...83,0 mg/L, the duration of cultivation 120 hours. Yeast cells in amount of 5%, $2x10^6$ cells/ml were inoculated in liquid medium.

Methods. The content of β-carotene in the yeast biomass was determined by spectrophotometric techniques as described (Tamaș et al., 1986). Catalase activity was determined by the method proposed by (Efremova et al., 2013). Statistical processing of results was done using Stata statistical software kit 7 that presents universal kit for solving of statistical problems in different fields, such as economy, medicine, biology. Veracity was compared to the control $p \leq 0.05$.

RESULTS AND DISCUSSIONS

Analysing the obtained results, it was found that effects of Fe_3O_4 and TiO_2 nanoparticles on β-carotene content and antioxidant enzyme catalase activity depend on concentration and dimension. The statistical analysis of the results has evidenced that β-carotene content was decreased with 34.5...73.4% compared to control sample at the cultivation in the presence of Fe_3O_4 nanoparticles with a dimension of 10 nm and reduced with 36.4...81.8% in case of utilization of Fe_3O_4 nanoparticles with the dimension of 30 nm (figure 1). The correlation analysis has been carried out to establish the role of nanoparticles in the process of β-carotene biosynthesis. The correlation report confirmed the dependence between β-carotene content and concentration of Fe_3O_4 (10 and 30 nm)

nanoparticles used at the cultivation of *Rhodotorula gracilis* CNMN-Y-30 strain that indicated a significant association between the two variables. The coefficient of determination was $R^2 = 0.928$ or 92.87 % for Fe_3O_4 (30 nm) nanoparticles and $R^2 = 0.911$ or 91.12 % for Fe_3O_4 (10 nm). The research results allowed to conclude that the addition of nanoparticles to cultivation medium could affect cellular membranes that are involved in β-carotene synthesis. This study has demonstrated that toxicity of nanoparticles depends on the dimension and concentration of NPs.

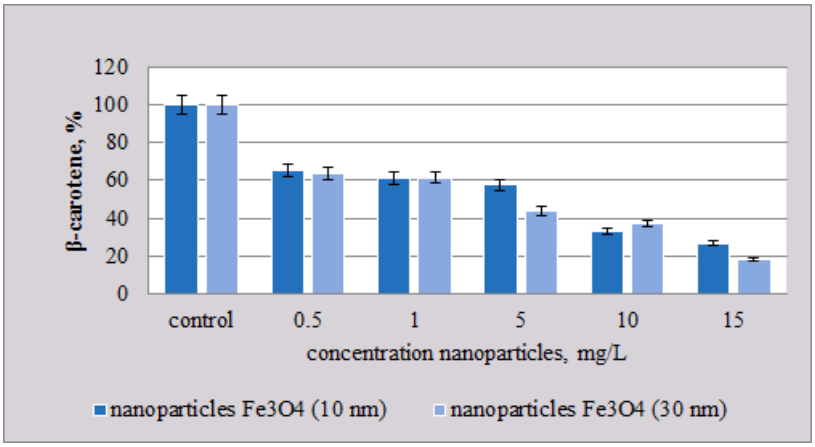

Figure 1. β-carotene content in biomass of *Rhodotorula gracilis* CNMN-Y-30 yeast strain at the cultivation in the presence of Fe_3O_4 nanoparticles with different dimensions.

Catalase activity in *Rhodotorula gracilis* CNMN-Y-30 yeast strain under the influence of Fe_3O_4 nanoparticles was subsequently studied. Catalase, acting as the first line of defense against reactive oxygen species, serves to neutralize free radicals, to protect cells from oxidative damage and toxic impact of different harmful factors. In the figure 2 below it is observed that the adaptive system of yeast cells was affected at the cultivation in presence of nanoparticles. Catalase activity in control sample constituted 29,5 mmol/min/mg protein, catalase activity values in experimental variants varied from 5.3 to 19 mmol/min/mg protein, representing the reduction of the enzyme activity with 17.1...61.3% compared with control for Fe_3O_4 (30 nm) nanoparticles and 4.6 to 52.3% for Fe_3O_4 (10 nm), respectively. It was established that concentration of nanoparticles has impact on catalase activity. The coefficient of determination constituted $R^2 = 0.930$ or 93.0% for Fe_3O_4 (30 nm) and $R^2 = 0.917$ or 91.7 % for Fe_3O_4 (10 nm), respectively. High concentrations of nanoparticles that caused significant modification of catalase activity could provoke the accumulation of hydrogen peroxide toxic for cell. Therefore, catalase activity is a potential indicator of the adaptive response formation of yeast strain.

Further research was carried out with TiO_2 nanoparticles with a dimension 30 nm. During the study of pigmentation rate of yeast biomass after 120 hours of submerged cultivation it was found that pigmentation was significantly modified compared to control at the addition of nanoparticles in concentration from 0.5 to 15 mg/l to culture medium (figure 3). Titanium dioxide nanoparticles have had negative impact on biosynthesis of carotenoid pigments regardless of used concentration. It was established that the reaction of response of the selected strain was manifested by the decrease of β-carotene content with 23.1 to 69.9% compared to control (figure 4). The strength of correlation was measured by the correlation coefficient, $R^2 = 0.9511$ or 95.11% to establish the interrelation between concentration of added nanoparticles and values of β-caroten content. High degree of association between two variables on the base of regression equation allows to predict the values of each other.

The analysis of obtained data on the influence of TiO_2 nanoparticles on catalase activity has revealed that low concentrations 0.5; 1.0 and 5.0 mg/L, added to cultivation medium, practically did not modified enzyme activity. The increase of nanoparticles concentration from 10.0 to 15.0 mg/L contributed to the decrease in catalase activity in experimental variants representing 87-70,9 % compared to control (figure 5).

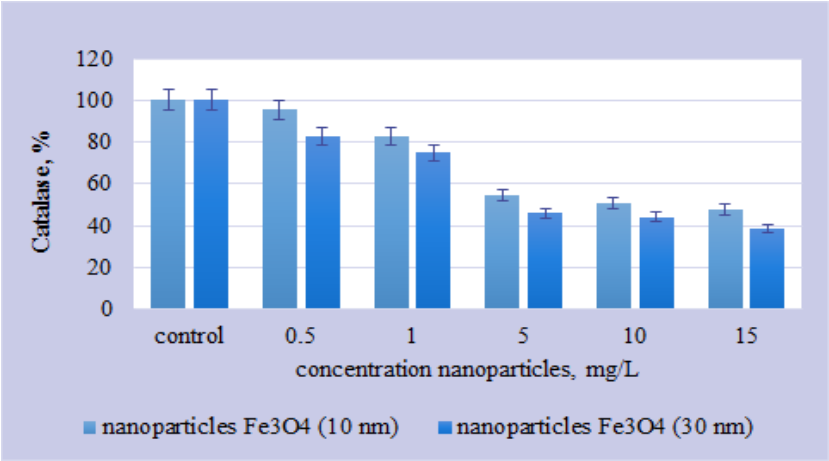

Figure 2. Catalase activity in biomass of *Rhodotorula gracilis* CNMN-Y-30 yeast strain at the cultivation in presence of Fe_3O_4 nanoparticles with different dimensions.

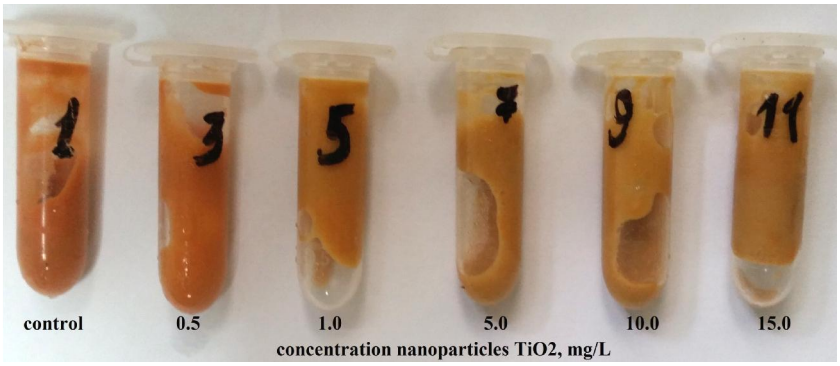

Figure 3. Influence of TiO_2 (30 nm) nanoparticles on pigmentation of biomass *Rhodotorula gracilis* CNMN-Y-30

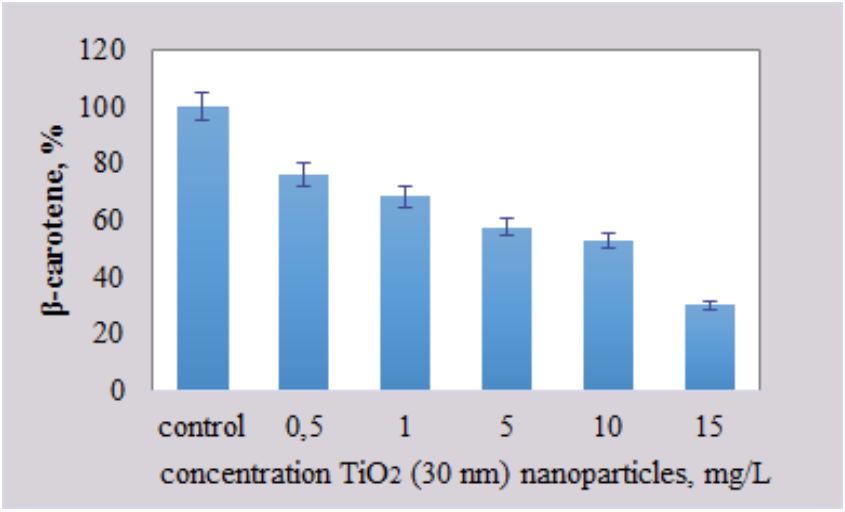

Figure 4. β-carotene content in biomass of *Rhodotorula gracilis* CNMN-Y-30 at the cultivation in the presence of TiO_2 (30 nm) nanoparticles depending on concentration.

Results of present study suggest that in case of supplementation of culture medium with TiO_2 nanoparticles the catalase activity was modified depending on used concentration of nanoparticles. A moderate correlation R^2 = 0.6236 was established as a result of evaluation of interrelation between concentration of nanoparticles and catalase activity at *Rhodotorula gracilis* CNMN-Y-30 yeast strain. The correlation report has demonstrated that TiO_2 nanoparticles affects biosynthetic processes at yeasts.

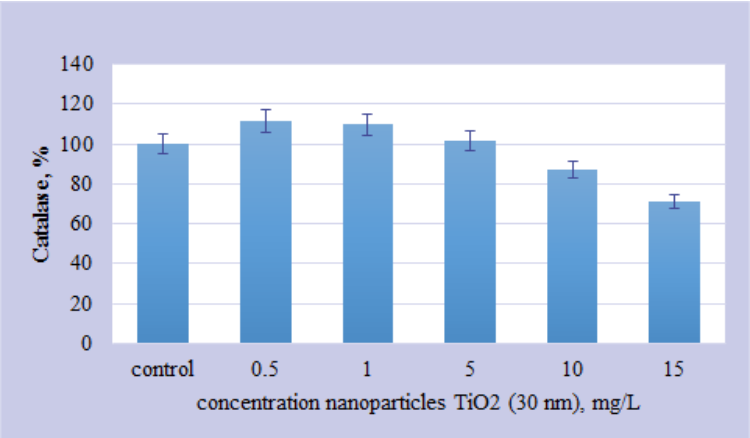

Figure 5. Catalase activity in biomass *Rhodotorula gracilis* CNMN-Y-30 at the cultivation in presence of TiO_2 (30 nm) nanoparticles depending on concentration.

CONCLUSION

Generalizing experimental results, it can be mentioned that adaptive response of pigmented yeast strain *Rhodotorula gracilis* CNMN-Y-30 to the presence of Fe_3O_4 (10 and 30 nm) and TiO_2 (30 nm) nanoparticles induced modification of the processes of β-carotene biosythesis and catalase activity. Thus, Fe_3O_4 and TiO_2 nanoparticles have induced the significant decrease in β-carotene content at *Rhodotorula gracilis* CNMN-Y-30 yeast strain. The big interval of variation can be explained by the fact of toxicity of nanoparticles used in this study. It can be mentioned that toxic effect of nanoparticles has grown with the increasing of concentration. The utilization of Fe_3O_4 nanoparticles contributes to the decrease in catalase activity. At the same time, supplementation of culture medium with TiO_2 nanoparticles in concentration from 0,5 to 5 mg/l leads to the stimulating of catalase activity. The increase of antioxidant enzyme activity enhanced oxidative stress tolerance of yeast cells.

Stress condition induced by nanoparticles selected for study has changed the content of studied parameters that take part of antioxidant defence system of cell. High degree of correlation between concentration of nanoparticles and β-carotene content, catalase activity was established at selected yeast strain. The correlation coefficient varied within limits $R^2 = 0.623$ to 0.951. The obtained results would contribute to the development of theoretical basis for nanotoxicological studies.

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