# THE EFFECT OF ZnO NANOPARTICLES ON THE ACTIVITY OF ANTIOXIDANT ENZYMES AND CAROTENOID CONTENT AT *RHODOSPORIDIUM TORULOIDES* CNMN-Y-30 YEAST

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**Keywords:** yeast, ZnO nanoparticles, *Rhodosporidium toruloides,* carotenoids, catalase, superoxide dismutase.

**Abstract**: The present research paper provides new information on the influence of ZnO nanoparticles (ZnO NPs) of size <50 nm and <100 nm on *Rhodosporidium toruloides* CNMN-Y-30 pigmented yeast. It was established that the activity of antioxidant enzymes such as catalase, superoxide dismutase and content of carotenoid pigments in the studied strain has been modified depending on the size and concentrations of NPs. There were no significant differences between the activity of antioxidant enzymes and content of carotenoid pigments in experimental group and control at the use of significantly low concentration of ZnO NPs. The use of nanoparticles in concentration of 30 mg/l caused a decrease in activity of antioxidant enzyme catalase and contributed to the increase in the activity of superoxide dismutase. This study has revealed that the concentration of 30 mg/L of ZnO NPs initiates an significant decrease in the content of carotenoid pigments - β-carotene, torulene and torularhodin in cell biomass. The results provided opportunities for modeling cell cycle processes and highlighting of carotenoid pigments and antioxidant enzymes as parameters for determining the mode of action of nanoparticles.

## INTRODUCTION

Over the last decade, the development of science and technology is characterized by intensive study of the properties of nanoparticles and the elaboration different methods for practical application of NPs. Zinc oxide nanoparticles are among the most widely used because of large total surface area, UV absorption property and chemical stability (Dastjerdi et al., 2010; Osmond et al., 2010; Bernhardt et. al, 2010; Gunawan et al., 2013). ZnO NPs are widely used in various applications including food industry. ZnO nanoparticles can be used in food packaging as antimicrobial agents to prevent contamination of foods (Sirelkhatim et al., 2015) or UV light absorbers to protect foods that are sensitive to UV light exposure (EFSA., 2016). Wide range of application of ZnO nanoparticles in the food industry highlights the problem to provide consumers a safe and contamination free food.

Numerous *in* vitro studies have demonstrated the toxic effect of ZnO nanoparticles, such as induction of oxidative stress, cell apoptosis, cytotoxic, genotoxic and inflammatory responses (De Angelis et al., 2013; Johnson et al., 2015; Song et al., 2010; Wahab et al., 2014; Cheng et al., 2017). The cytotoxicity of nanoparticles and their interaction with biological systems is still unclear. Thus, there is a need for studies that contribute to our understanding of the mechanism of influence of nanoparticles and to evaluate the risk of utilization of them. Therefore, the possible adverse effects of exposure to nanoparticles were studied on different organisms (Bhuvaneshwari et al., 2015; Hazeem et al., 2016; Prach et al., 2013). Yeasts *Rhodotorula gracilis* can serve as biological model for studying the influence of nanoparticles on molecular and cellular mechanisms. According to recent publications, scientific name *Rhodosporidium toruloides* is the synonym of *Rhodotorula gracilis*. The structural and functional organization of yeast cell, ability to biosynthesize a wide range of antioxidants position this unicellular eukaryotic organism as a model for evaluating of adaptive cell response to nanoparticle action.

The evaluation of antioxidant potential is important to determine the action of nanoparticles on yeasts cells. According to the scientific literature, reactive oxygen species (ROS) production induced by nanoparticles in high concentrations , affect cell physiology, causing DNA and cell membrane damage (Jaleel et al., 2008; Puja et al., 2015). Cells possess the antioxidant defense system that can prevent ROS mediated damage (Zannatul et al., 2009; Rahman et al., 2016). Antioxidant enzymes, including catalase and superoxide dismutase and carotenoid pigments form the first line of defence against free radicals in *R. gracilis* yeast strains.

Thus, the research aim was to evaluate modifications in the activity of intracellular antioxidant enzymes and carotenoid pigments in *R. toruloides* CNMN-Y-30 yeast strain under the influence of ZnO nanoparticles depending on size and concentration.

## MATERIALS AND METHODS

*Objects of study.* Pigmented yeast strain *Rhodosporidium toruloides (synonim Rhodotorula gracilis)* CNMN-Y-30, producer of proteins and carotenoids, was selected for the research (Usatîi et al., 2016). 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 Moldova.

*Nanoparticles:* ZnO nanoparticles with particle size <50 nm in form of nanopowder, purity >97%, contains 6% Al dopant, surface area >10,8 $m^2$/g (ALDRICH) and with size <100 nm in form of nanopowder, purity >80%, surface area 15-25 $m^2$/g (ALDRICH) were used. The suspension of nanoparticles was prepared according to the method specified (Oterro-Gonzalez et al., 2013). The concentrations of nanoparticles used in experiments constituted 1.0; 5.0; 10; 15; 20; 25; 30 mg/L. The variant without application of nanoparticles was used as control sample.

*Culture media:* YPD media specific to yeast strains was used for the cultivation (Agiular et al., 2003). The submerged cultivation was carried out in depth capacity 1 liter Erlenmeyer flasks on shaker 200 rpm at a temperature of $25^0$...$28^0$ C, the duration of cultivation 120 hours. Yeast cells in amount of 5%, 2x106 cells/ml were inoculated in liquid medium. The cultivation of yeasts was effectuated at the constant illumination of 2000 Lx.

*Methods.* Catalase activity was determined by method Aebi (1984) modified by Efremova et al. (2013). Superoxide dismutase activity was determined on PG T60 VIS Spectrophotometer, at 560 nm, according to method described by (Некрасова et al., 2008). The carotenoid content in the yeast biomass was determined by spectrophotometric techniques described by (Frengova et al., 1994; El-Banna Amr et al., 2012; Tămaş et al., 1986). Statistical analysis of results was done using computerized application statistics 7 for windows.

## RESULTS AND DISCUSSIONS

An important indicator of cellular resistance under stress conditions is the activity of antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD) (Fridovich., 1995). Catalase is known for its ability to convert toxic hydrogen peroxide into water and oxygen. Antioxidant enzymes play a major role in reducing ROS levels. The increase in superoxide dismutase and catalase activities could be an adaptive response to the action of nanoparticles on yeasts cell. The determination of antioxidant enzymes activity of yeast *R. toruloides* CNMN-Y-30 under the influence ZnO (<50 nm) and (<100 nm) nanoparticles and demonstrated that at the use of low concentration of NPs (1-5 mg/L) the catalase activity in experimental samples was similar to the control (Figure 1). However, if the concentration of nanoparticle was increased to 30 mg/L, the activity of the catalase was reduced, so cell exhibited a diminished ability to protect themselves from consequences of oxidative stress and accumulation of hydrogen peroxide. No significant correlation between the nanoparticle concentrations and the activity of the catalase was established, $R^2 = 0.7118$ for ZnO (<50 nm) nanoparticles and $R^2 = 0.2176$ for ZnO (<100 nm) nanoparticles (Figure 1).

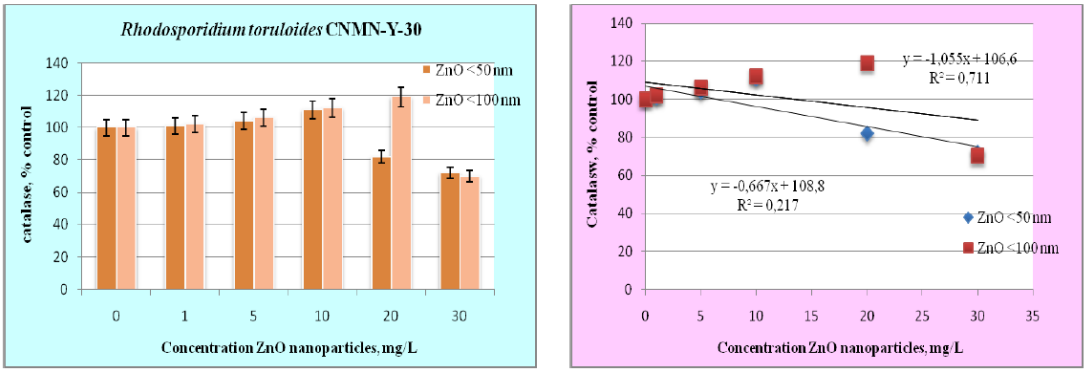


Figure 1. Catalase activity at *Rhodosporidium toruloides* CNMN-Y-30 under the action of ZnO nanoparticles depending on size and concentration

The results of the study of superoxide dismutase activity in *R. toruloides* CNMN-Y-30 under the influence of ZnO (<50 nm) and (<100 nm) nanoparticles at the concentrations from 5 mg/L to 30 mg/L revealed a trend towards an increase in the experimental samples (Figure 2). SOD increased significantly by 53% for ZnO (<50 nm) NPs and 85% for ZnO (<100 nm) NPs at the concentration of 20 mg/L. The values of superoxide dismutase activity in the control yeast biomass constituted 132...135 U/mg protein. ZnO (<50 nm) nanoparticles and ZnO (<100 nm) nanoparticles at the concentration of 20 mg/L caused an increase in the activity of SOD up to 202 U/mg protein and 250 U/mg protein. So, ZnO nanoparticles significantly affected the antioxidant enzyme system of studied yeast strain. Analysing the action of ZnO nanoparticles on SOD activity, it can be mentioned that concentrations of NPs correlate to 79% for ZnO (<100 nm) nanoparticles and practically does not depend on used concentrations for those of ZnO (<50 nm) nanoparticles, $R^2$ = 0 ,3667 (Figure 2).

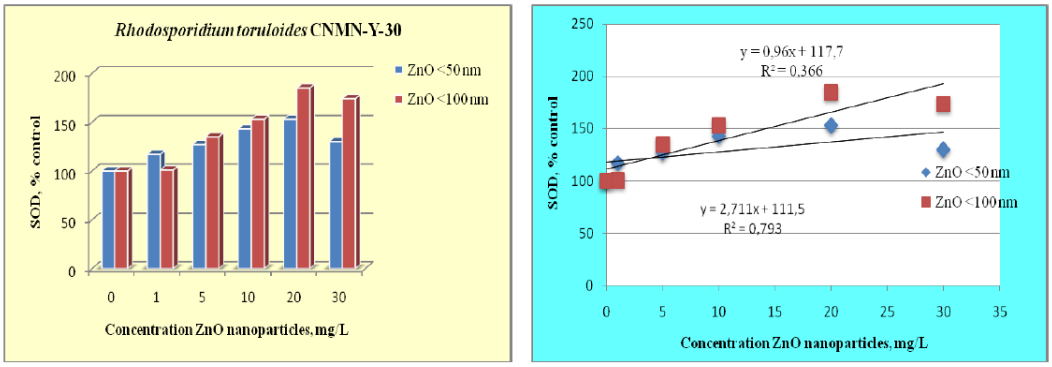


Figure 2. Superoxide dismutase activity at *Rhodosporidium toruloides* CNMN-Y-30 under the action of ZnO nanoparticles depending on size and concentration

Various studies have elucidated first line antioxidant defense mechanisms (Jamec., 2008; Yang ., 2009; Lassegue., 2010). There are a number of antioxidants that act to prevent the formation of reactive species in cells, others scavenge free radicals and inhibit pro-oxidants (Carocho et al., 2013). Carotenoid pigments also known as macromolecular antioxidants, play an important role in protecting cells from oxidation and cellular damages. Carotenoids are recognized as singlet oxygen collectors. Thus, in the conditions of oxidative stress, these pigments can be used as biological markers for assessing the degree of influence of metal oxide nanoparticles on living systems.

Therefore, to understand the effects of ZnO nanoparticles on *R. toruloides* CNMN-Y-30 yeast depending on size and concentration, it is essential to study the formation of carotenoid pigments, including β-carotene, torulene and torularhodin. So, its was established that low concentrations of ZnO (<50 nm) nanoparticles do not deviate significantly process of biosynthesis of carotenoids. A modest increase (18%) of the carotenoids content was observed in the case of utilization of 10 mg/L of nanoparticles. In other experimental variants, the content of pigments in yeast biomass do not differ from the control (Figure 3). At the same time, the statistical analysis of results of the influence of ZnO (<100 nm) NPs on yeasts highlighted that supplementation of the culture medium with 30 mg/L of nanoparticles initiates an essential (36%) decrease of the amount of carotenoid pigments in the yeast biomass. Thus, large size and high concentrations of nanoparticles can alter the biosynthesis process of carotenoid pigments. So, free radicals in excess can damage cell membranes responsible carotenoid biosynthesis. The dependence of the concentrations of ZnO (<100 nm) nanoparticles and carotenoid content is expressed by the coefficient of determination $R^2 = 0.5622$. Therefore, the concentration of nanoparticles determines the accumulation of carotenoid pigments in only 56% of cases. In the case of the use of ZnO (<50 nm) nanoparticles, the concentration determines the accumulation of carotenoid pigments in 16% of cases (Figure. 3).

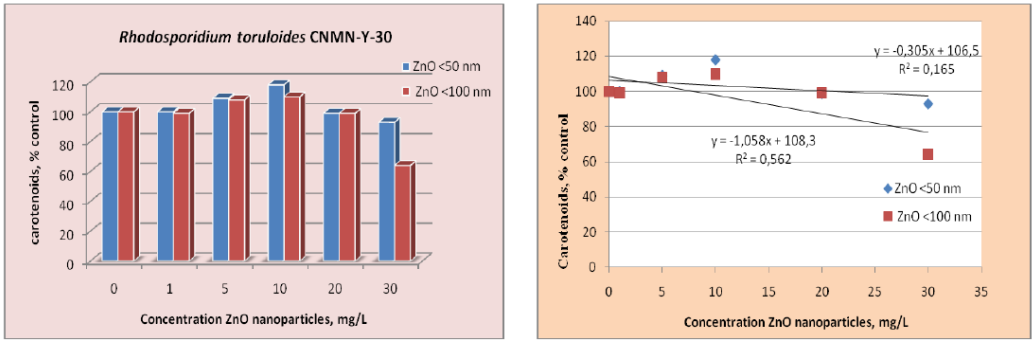


Figure 3. The content of carotenoid pigments at *Rhodosporidium toruloides* CNMN-Y-30 under the action of ZnO nanoparticles depending on size and concentration

According to recent studies on *Rhodosporidium* genus, proposed pathways for carotenoid biosynthesis including beta-carotene, torulene and torularhodin synthesis depend on the properties of used yeast strain and cultivation conditions (Singh et. al, 2016; Merhan**.,** 2017). In view of the fact that carotenoid content was influenced by the application of ZnO nanoparticles and some quantitative variations were established against the control samples, it was studied the accumulation of basic components of pigments.

The obtained results have demonstrated that, the proportions of β - carotene, torulene and torularhodin does not change significantly under the influence of ZnO (<50 nm) nanoparticles (Figure 4). This phenomenon indicates that nanoparticles in most used concentrations do not affect pathways for carotenoid biosynthesis.

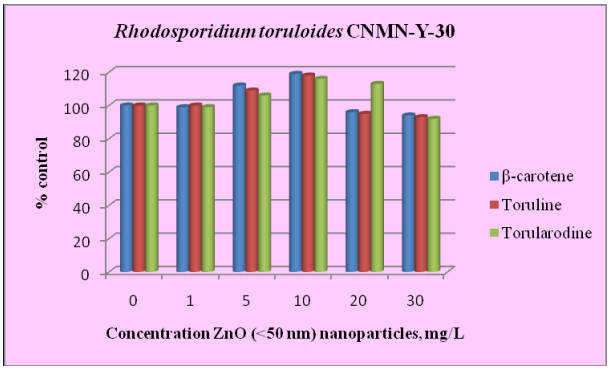


Figure 4. The level of β-carotene, torulene and torularhodin at *Rhodosporidium toruloides* CNMN-Y-30 under the action of ZnO (<50 nm)nanoparticles

Analysing the content of the basic components of carotenoid pigments under the influence of ZnO (<100 nm) nanoparticles some changes depending on the applied concentrations were established. β-Carotene, one of the indices of responses of the yeast cell, at concentration of 30 mg/L, was decreased by 33% and the content of torulene decreased further by 51% (Figure 5). Thus, the obtained results suggest that large size nanoparticles, upon contact with the surface of the yeast cells, can cause some metabolic disorder, thus modifying the specific biochemical reaction. This information provides opportunities in modeling vital cell processes and highlighting carotenoid pigments as a model for determining the degree of action of nanoparticles.

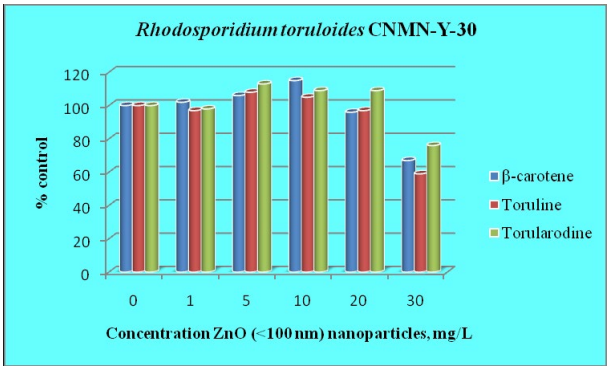


Figure 5. The level of β-carotene, torulene and torularhodin at *Rhodosporidium toruloides* CNMN-Y-30 under the action of ZnO (<100 nm) nanoparticles

## CONCLUSIONS

Generalizing the results obtained in this study it can be mentioned that the activity of the antioxidant enzymes catalase, superoxide dismutase and the content of carotenoid pigments at *Rhodosporidium toruloides* strain CNMN-Y-30, in contact with the ZnO nanoparticles was changed depending on the size and applied concentration. Estimating the degree of influence of the ZnO nanoparticles (<50 nm) and ZnO (<100 nm) on the yeast *Rhodosporidium toruloides* CNMN-Y-30 was established that, in the case of low concentrations of nanoparticles (1-5 mg/L), the activity of antioxidant enzymes and content of carotenoid pigments in the experimental samples was similar to the control. Nanoparticles examined at a concentration of 30 mg/L caused the decrease in carotenoid content, the activity of the antioxidant enzyme catalase and the increase of superoxide dismutase activity. Changes in the biosynthesis of carotenoid pigments expressed by the reduction of β-carotene, torulene and torularhodine associated with the decrease in the catalase activity provide opportunities for modeling of vital processes and revealing carotenoids and antioxidant enzymes as biological test in terms of determining the degree of action of nanoparticles on the yeasts.

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