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SHORT COMMUNICATION

THE INFLUENCE OF ZNO NANOPARTICLES ON THE AMINO ACIDS BIOSYNTHESIS AT PIGMENTED YEAST *RHODOTORULA GRACILIS* CNMN-Y-30

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Abstract: The research paper provides data about the influence of ZnO nanoparticles (NPs) of size <50 nm on the content of proteins and amino acids at pigmented yeast strain *Rhodotorula gracilis* CNMN-Y-30. The results have highlighted that concentrations of 1-20 $mg \cdot L^{-1}$ present an opportunity for application in the technology of cultivation of yeast studied, in terms of increasing the protein quantities by with up to 38 %. Also essential, immunoactive and proteinogenic amino acid content measured in yeast biomass after exposure to ZnO nanoparticles in concentrations of 20-70 $mg \cdot L^{-1}$ they increased significantly compared to control values. Thus, it can be concluded that the proteins and amino acid content in the studied strain has been modified depending on the concentrations of NPs.

Keywords: *amino acid, protein content, Rhodotorula gracilis, ZnO nanoparticles, Yeasts cultivation*

INTRODUCTION

Nowadays, researches for the development of biotechnologies for the production of microbial proteins and amino acids are of great importance. The protein content characterizes the conduct of the metabolic processes of the crops subjected to the action of different cultivation factors. Consisting exclusively of amino acids, proteins are found in cells alongside other important cell components. Proteins can be enzymes that catalyze various biochemical reactions in the body, and others can play an important role in maintaining cellular integrity (cell wall proteins), in the immune and autoimmune response of the organism in the language, structure and cellular functioning [1]. The final use of proteins is extremely broadly expanded with numerous industrial and commercial applications [2 – 5]. Industrial demand is on a continuous growth led by the growing need to find sustainable solutions for production of microbial proteins.

An innovative concept in the biotechnological production of microbial proteins is the use of zinc oxide nanoparticles as stimulating and regulatory factors of biosynthesis processes [6 – 8]. The nanoparticles present a unique tool for handling the biosynthetic activity of micro-organisms, with proven efficiency on biotechnological objects in different taxonomic groups. The stimulating impact of zinc oxide nanoparticles is due to the unique properties of interacting with microbial cells and inducing changes in cellular biochemical processes, including protein components.

A biotechnological object of great perspective represent the pigmented yeasts of the genus *Rhodotorula gracilis* that can produce large quantities of proteins with up to 30 % of their dry biomass, and after the controlled cultivation they can register up to 40 % [9, 10].

Thus, the aforementioned indicate the importance of evaluating the influence of ZnO NPs on yeast strains of the *Rhodotorula* genus and assessing the potential for use in biotechnology for enhancing protein and amino acids synthesis. Investigations in this field are both theoretical and practical.

MATERIALS AND METHODS

Objects of research. The strains *Rhodotorula gracilis* CNMN-Y-30, a carotenoid protein and pigment producer, served as study objects.

Nanomaterials. In the experiments ZnO nanoparticles < 50 nm in the form of powder (ALDRICH), chemical synthesis, purity > 90 %, contains 6 % Al dopant, surface area >10.8 $m^2 \cdot g^{-1}$. The nanoparticle stock solution was prepared according to the method outlined by [11]. ZnO nanoparticles were added to the nutrient medium in emulsion form at the inoculation stage of the studied strains.

Culture Media. YPD (yeast-peptone-dextrose) fermentation medium and wort must have been used to obtain the seed material and to grow the yeasts *Rhodotorula gracilis* [12].

Submerged cultivation will be carried in Erlenmeyer flasks 1.0 L, the rotating speed of the stirrer 200 rpm, at 25 - 27 ^{o}C, the degree of aeration 80.0 - 83.0 $mg \cdot L^{-1}$, permanent lighting 2000 Lx, the time of cultivation 120 hours. Broth medium will be seeded in an amount of 5 % with the inoculum 2 x 106 $cells \cdot mL^{-1}$.

Methods of achieving research. The protein was determined by spectrophotometric method based on protein extraction with 0.1N NaOH using standard bovine serum albumin as a standard sample [13]. The composition of amino acids in the yeast strain *Rhodotorula gracilis* CNMN-Y-30 was analyzed by acid hydrolysis using the "AAA-339" analyzer of the "Microtehna" Company (Czech Republic) [14].
The statistical analysis of the results was carried out using the set of MO Excel programs and Statistics 9.0. The data results of 3 - 5 repetitions obtained were expressed by calculating the mean, standard deviation and confidence interval for an average. All differences were considered statistically significant for $P \leq 0.05$.

RESULTS AND DISCUSSION

Identification of the character of action of the ZnO (< 50 nm) nanoparticles on the biosynthesis process of proteins in the *Rhodotorula gracilis* CNMN-Y-30 strain cultivated on the YPD environment revealed that their content in cellular biomass, under the influence of concentrations of nanoparticles from 5 to 30 mg·L^{-1} is increasing by 9 - 38 %, compared to the blank sample (Figure 1). In experimental samples in which the concentration of nanoparticles constituted 50 - 70 mg·L^{-1}, there was a relatively moderate decrease in the protein content to studied yeast (Figure 1). The determination of the correlation between the amount of yeast biomass proteins and the concentrations of ZnO (< 50 nm) nanoparticles used in experiments demonstrated a strong association, R^2 =0.8426 for the strain *Rhodotorula gracilis* CNMN-Y-30. Based on the above, it has been established that effective concentrations of ZnO (< 50 nm) nanoparticles to stimulate the protein content of the studied yeast are 5 - 30 mg·L^{-1}.

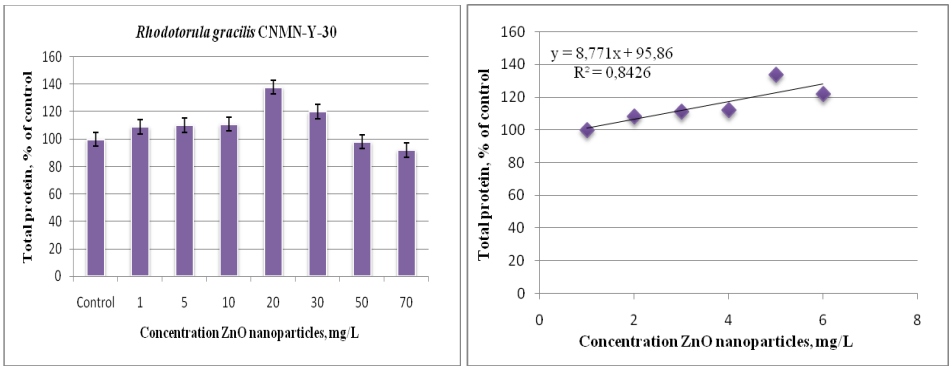

Figure 1. *The influence of ZnO (< 50 nm) nanoparticles on protein content to pigmented yeasts Rhodotorula gracilis CNMN-Y-30*

These results are consistent with other scientific studies demonstratingthat exposure of *P. stratiotes* plants to metallic NPs (copper and zinc) has been proven to increase protein content in experimental variants (Figure 2) [15]. According to data, about 40 % of all known proteins contain metal cations, probably Zn^{2+} ions bind to proteins [16 - 19]. Binding of proteins to ZnO nanoparticles induces changes by improving and diversifying their function.

Next, in order to establish the influence of ZnO NPs on the quality of yeast proteins, the amino acid composition was analyzed. Examination of the quantitative and qualitative composition of yeast *Rhodotorula gracilis* CNMN-Y-30 proteins, when cultured on YPD nutrient medium, showed a high content of essential amino acids 6.0234 mg/100 mg, immunoactives 5.64 mg/100 mg and proteinogens 12.45 mg/100 mg (Figure 2). From the spectrum of the 10 essential amino acids in the yeast composition, 7 were identified: threonine, valine, methionine, isoleucine, leucine, phenylalanine, lysine and 2 semi-essential amino acids histidine and arginine. Also 8 immunoactive amino acids that are part of the composition of immunoglobulins and participate in the processes of protection of the body [18, 20] aspartic acid, glutamic acid, alanine, cysteine, glycine, serine, valine and arginine. Also recent studies have reported the role of amino acids as promising agents in the management of proliferative metabolism, and the application of amino acids has been rapidly increased in a variety of therapeutic fields [21, 22].

When culturing the yeast in the presence of ZnO (< 50 nm) nanoparticles in concentrations of 20 and 70 $mg \cdot L^{-1}$, a quantitative increase of amino acids was found compared to the control sample. The sum of essential amino acids in yeast biomass was 13.31 mg/100 mg, and 16.8307 mg/100 mg, respectively, being 120 – 179 % higher than the control variant. It should be mentioned that in the share of essential amino acids a considerable contribution is made by histidine, isoleucine, leucine, lysine and arginine (Figure 3).

Determining the degree of correlation between the amount of essential amino acids and the concentration of ZnO nanoparticles has shown a strong link. The coefficient of determination is 0.961 or 96 % confirms the existence of a true dependence between these two parameters.

The quantitative values of the immunoactive amino acids increased significantly and constitute 10.87 - 18.98 mg/100 mg, exceeding the control variant by 92 – 236 %. The correlation between the amount of immunoactive amino acids and the concentration of ZnO nanoparticles has shown a strong link. The coefficient of determination is $R^2 = 0.882$.

Representative stimulation of the of amino acids especially aspartic/glutamic acid, threonine, leucine, lysine, arginine and histidine (Figure 2) can be explained by the intracellular metabolic response of yeast *Rhodotorula gracilis* CNMN-Y-30 to the stress caused by the application of high concentrations of ZnO (<50 nm)NPs, significant effect is observed at the applying the concentration of 70 $mg \cdot L^{-1}$. The results obtained in Figure 2 are consistent with data from the literature according to which the interaction of metallic NPs with plants and microorganisms induces stress and led to the acceleration of synthesis of various metabolites, including amino acids [15, 23].

It has also been reported that ZnO nanoparticles stimulate the biosynthesis of amino acid content compared to other metal nanoparticles such as CuO. Comparative analysis of the effect of metallic NPs demonstrated that the total amino acid content of *P. stratiotes* plants is 88.2 $\mu mol \cdot g^{-1}$ under the influence of copper NPs and 101.5 $\mu mol \cdot g^{-1}$ under the influence of ZnO NPs, thus indicating a stronger negative effect of copper compared to ZnO NPs [15].

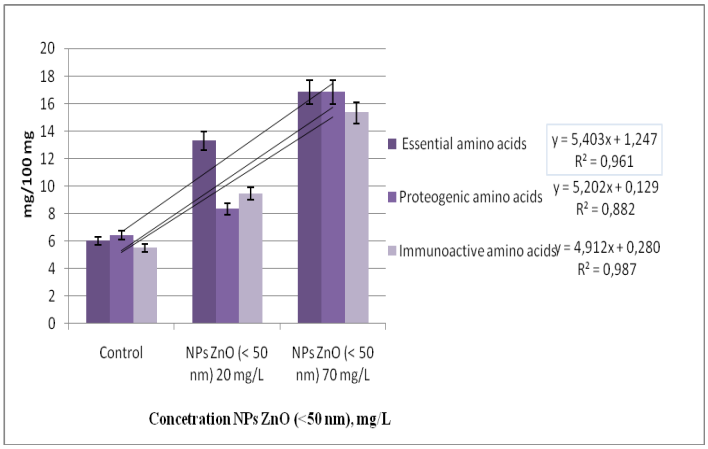

Figure 2. *The content of amino acids in Rhodotorula gracilis CNMN-Y-30 biomass, cultivated in the presence of ZnO nanoparticles*

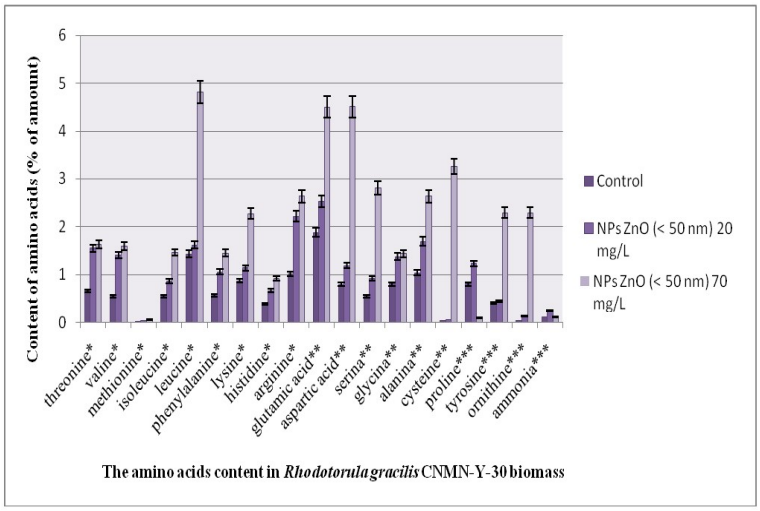

Figure 3. *Quantitative and qualitative amino acid composition (essential*, proteogenic** and immunoactive***) in Rhodotorula gracilis CNMN-Y-30 biomass, cultivated in the presence of ZnO nanoparticles*

CONCLUSIONS

Generalizing the results obtained in this study it can be mentioned that the effect of ZnO (< 50 nm) nanoparticles on pigmented yeasts of the genus *Rhodotorula* develops according to the used concentrations.

Concentrations of 10 - 20 $mg \cdot L^{-1}$ present an opportunity for application in the technology of cultivation of yeasts *Rhodotorula gracilis* CNMN-Y-30, in terms of increasing the protein quantities by 9 - 38 %, compared to the control. Increasing the concentrations of the ZnO nanoparticles up to 50 - 70 $mg \cdot L^{-1}$, is the condition for the inhibition of, protein biosynthesis processes.

Concentrations of ZnO nanoparticles of 20 - 70 mg·L^{-1}, initiate the increase of the amounts of essential amino acids by 120 - 179 %, immunoactive by 92 - 236 % and proteinogenic by 50 - 177 %, compared to the control.

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