Analele Științifice ale Universității „Alexandru Ioan Cuza”, Secțiunea Genetică și Biologie Moleculară
TOM XVIII, Fascicula 2, 2017

THE ACTION OF T_iO_2, ZnO, Fe_3O_4 NANOPARTICLES ON *SACCHAROMYCES* AND *RHODOTORULA* YEAST STRAINS IN FUNCTION OF THE CONCENTRATION AND DIMENSIONS

AGAFIA USATÎI[1]*, NATALIA CHISELIȚA[1], LUDMILA BEJENARU[1], ALINA BEȘLIU[1], NADEJDA EFREMOVA[1], ELENA TOFAN[1]

Received: 14 February 2017 / Revised: 13 March 2017 / Accepted: 29 March 2017 / Published: 6 July 2017

Keywords: nanoparticles, *Saccharomyces*, *Rhodotorula*, proteins, carbohydrates, mannoproteins, carotenoid pigments.
Abstract: The present paper reports on influence of some nanoparticles of metal oxides on *Saccharomyces* and *Rhodotorula* yeast strains. It has been established that the main factors determining cell response to the action of metallic nanoparticles are concentration and dimension, as well as important indices of this adaptive reaction are proteins, carbohydrates, including β-glucans and mannoproteins, and carotenoid pigments content.

INTRODUCTION

Nanotechnologies represents an innovative scientific and economic domain. The unique properties and utility of nanoparticles (NPs) allow application in different fields such as biology, medicine, chemistry, physics etc. (Mrinmoy De et al., 2008, Vaseem et al., 2010, Potara, 2012, Gardikiotis, 2012, Espita et al., 2012, Kiran et al., 2014). It is considered that inorganic nanoparticles due to the new properties, could modify metabolic pathway of living organisms. The application of inorganic nanoparticles at the cultivation of microorganisms represents a recent research domain of nanobiotechnology (Nasr , 2015, Eden Mahendra, 2011). Recent studies have demonstrated that more frequent nanoparticles side effects on cells are: a) nanoparticles uptake by cells followed by the interruption of ATP production and DNA replication; b) the formation of reactive oxygen species (ROS), that leads to cell apoptosis; c) nanoparticles induced cell membrane damages (Sahayaraj, Rajesh, 2011). Insufficient amount of available data on the results of the evaluation of nanoparticles effect on living organisms that tended to be piecemeal has created difficulties of estimating the impact and risks of nanoparticles application in different domains.

Metallic nanoparticles are of great importance. The properties of nanoparticles could be magnetic, bactericidal and catalytic depending on metals. It's important to emphasis the effect of nanoparticles from the perspective of enhancing the technological properties for different opportunities. Biotechnological perspectives on the application of ZnO nanoparticles in different domains are mentioned in some publications (Espita et al, 2012; Vaseem et al., 2010). Comparative with other antimicrobial agents, TiO_2 NPs have attracted a lot of attention due to the high stability, absence of toxicity, low cost, bioactive activity. According to special literature data, minimum inhibitory concentration (MIC) of nanoparticles varies and depends on the microorganism selected for study (Pișkin et al., 2013). The possible mechanisms of TiO_2 action on cellular level have been investigated by other researchers (El-Said et al., 2014; Minju al., 2013) which have characterized some processes of nanoparticles application. The important properties of magnetic iron oxide nanoparticles (NPs) destined for biomedical and biotechnological application have been mentioned in scientific papers of some researchers (Hongtao Cui et al., 2013; Kiran et al., 2014). An important type of Fe_3O_4 nanoparticles is of great interest for biomedicine due to the superparamagnetism and biocompatibility (Guiden et al., 2004).

In the context of developing of technological possibilities of elaboration of new bioproducts for the utilization in different domains of national economy, it is important to study the nanoparticles effect on development and metabolites production at yeasts with biotechnological destination. Thereby, this work aims to determine the mechanism of action of nanoparticles on microorganisms by focusing of the effect of selected nanoparticles on processes affecting biosynthesis of cell components.

The propose for research important scientific problem consists in identification of *Saccharomyces* and *Rhodotorula* yeast response to the nanoparticles action of some metals depending on concentration and dimension for the following investigations regarding processes the biosynthesis modelation of bioactive principles of biotechnological interest.

MATERIALS AND METHODS

Strains, culture medium, cultivation conditions. The following yeast strains: *Saccharomyces cerevisiae* CNMN-Y-18 selected as mannoproteins producer (Usatîi et al., 2013), *Saccharomyces cerevisiae* CNMN-Y-20 as β-

glucans producer (Chiselița et al., 2010), *Rhodotorula gracilis* CNMN-Y-30 as carotenoids producer were used for the study.

TiO_2, ZnO, Fe_3O_4 nanoparticles elaborated by the researchers of the Institute of Electronic Engineering and Nanotechnologies of Academy of Sciences of Moldova (Guțul et al., 2014). Nanoparticles, besides magnetite, were stabilized by polyvinylpyrrolidone (PVP). Suspension of Fe_3O_4 nanoparticles was obtained using the method previously described by (Oterro-Gonzalez et al., 2013). Concentrations of nanoparticles used at the yeast cultivation varied depending on the aim of experiment. The variant without application of nanoparticles served as control sample.

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

Methods. Protein was determined spectrophotometrically by the Lowry method (Lowry et al., 1951) using bovine serum albumin as standard. The content of total carbohydrates in yeasts biomass was determined using PG T60 VIS spectrophotometer at wavelength of 620 nm with the utilization of anthron reagent and D-glucose as standard (Dey et al., 1993). The β-glucans content in the yeast biomass was determined gravimetrically as described (Thammakiti et al., 2004). Mannoproteins content was determined gravimetrically according to the method (Liu Hong-Zhi et al., 2009, Zhang et al., 1999). Carotenoids pigments were extracted from yeast biomass and measured spectrophotometrically (Frengova et al., 1994, El-Banna Amr et al., 2012, Tămaș et al., 1986). Statistical processing of obtained results was effectuated with set of programs Statistics 7.

RESULTS AND DISCUSSIONS

30 nm TiO_2 and 10 nm Fe_3O_4 nanoparticles were used to establish the role of the nanoparticles concentrations in the cultivation process of yeasts *Rhodotorula* and *Saccharomyces.* The modification of cultivation conditions of yeasts may induce the formation of reactive oxygen species. Carotenoids pigments being the essential components with free radical scavenging property have an important role in regulation of oxidative stress and its negative consequences. The role of carotenoids in adaptive cell reaction present a great interest. The statistical analysis of experimental data on influence of TiO_2 nanoparticles on *Rhodotorula gracilis* CNMN-Y-30 pigmented yeast strain have revealed the significant decrease of carotenoids content at the application of different nanoparticles concentrations. The correlation among pigments content and applied concentration of nanoparticles has demonstrated a strong association R^2 = 0,940 (Figure 1).

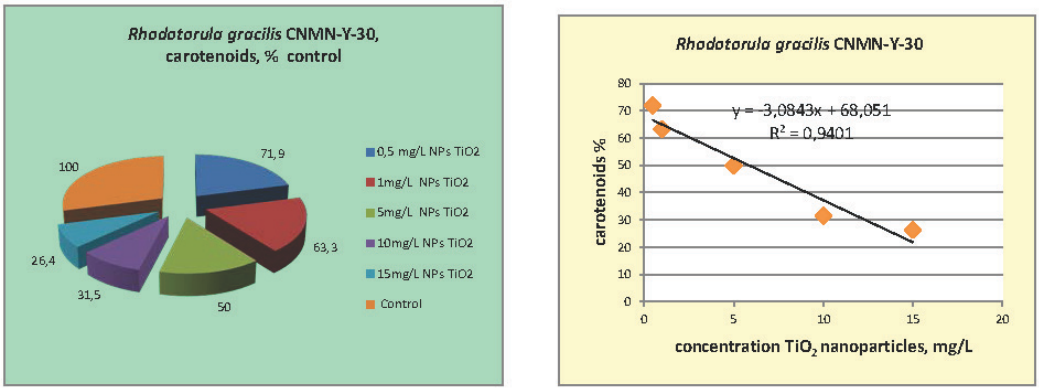

Figure 1. The influence of TiO_2 nanoparticles on carotenoids content in biomass *Rhodotorula gracilis* CNMN-Y-30 depending on concentration.

Under the influence of another type of Fe_3O_4 nanoparticles the β-carotene content, one of the base indices of adaptive response of pigmented yeast strain *Rhodotorula gracilis* CNMN-Y-30 was reduced depending on concentration with 34,5... 85,5%. Low concentrations of Fe_3O_4 nanoparticles are less active than concentrations that surpass the values of 10 mg/l. The correlation between the nanoparticles concentration and β-carotene content was evident and was expressed by coefficient of correlation $R^2 = 0,902$ (Figure 2).

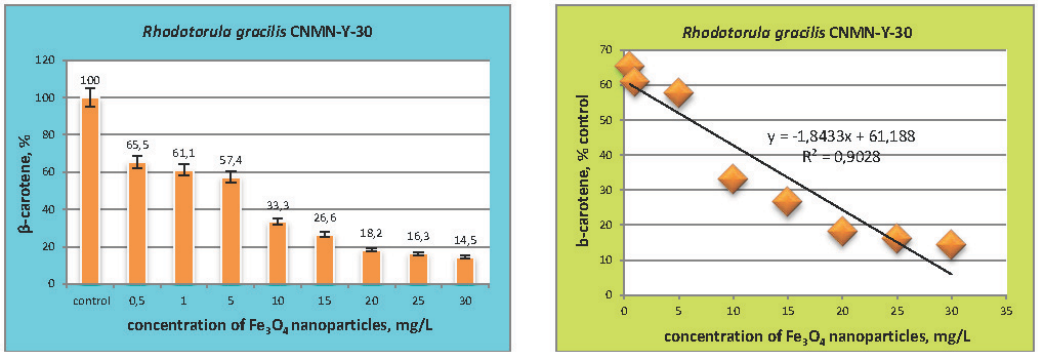

Figure 2. The influence of Fe_3O_4 nanoparticles on β-carotene content in biomass of *Rhodotorula gracilis* CNMN-Y-30 depending on concentration

The effect of TiO_2 nanoparticles on mannoproteins biosynthesis at *Saccharomyces cerevisiae* CNMN-Y-18 strain depending on concentration was significant, also. The obtained results have demonstrated that mannoproteins content increased with 11,9-22,6 % simultaneously with the nanoparticles concentration (Figure 3). The highest values of mannoproteins content were obtained at concentration limits 10-15 mg/L. This effect was caused by the property of yeast cells to uptake nanoparticles inducing redistribution of nutritive medium components and activation of carbohydrate metabolism. Thus, such an analysis of interrelation between the mannoproteins content and nanoparticles concentration can serve as a confirmation that has established a moderate ascending correlation ($R^2 = 0,556$), that argue the hypothesis of the existence of some interrelation in the base of which, according to the regression equation, could be prognosticated the selected indices.

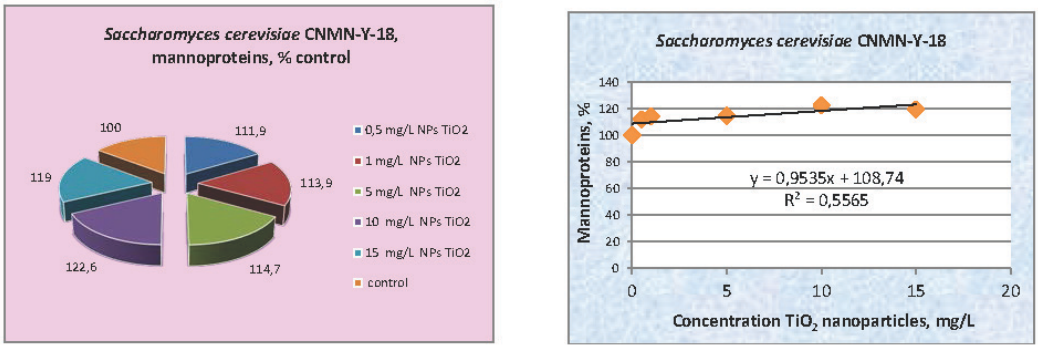

Figure 3. The effect of TiO_2 nanoparticles on mannoproteins accumulation at *Saccharomyces cerevisiae* CNMN-Y-18 strain depending on concentration.

Thereby, it can be affirmed that the study of influence of TiO_2 şi Fe_3O_4 nanoparticles on *Rhodotorula gracilis* CNMN-Y-30 and *Saccharomyces cerevisiae* CNMN-Y-18 strains has revealed that the nanoparticles effect was dependent on concentration that provided alterations of processes of bioactive principles biosynthesis depending on selected biotechnological object. TiO_2 and Fe_3O_4 nanoparticles have a high capacity to inhibit biosynthesis of carotenoid pigments and to induce the mannoproteins biosynthesis.

Another actual scientific problem was the study of peculiarities of nanoparticles action depending on dimension. The investigations devoted to the elucidation of the impact of ZnO nanoparticles on yeasts have demonstrated that the 30 nm dimensions have the capacity to stimulate the biosynthesis of proteins, carbohydrates, β-glucans at *Saccharomyces cerevisiae* CNMN-Y-20 yeast strain**,** qualities that did not manifest other nanoparticles with 10 nm dimensions (Figure 4).

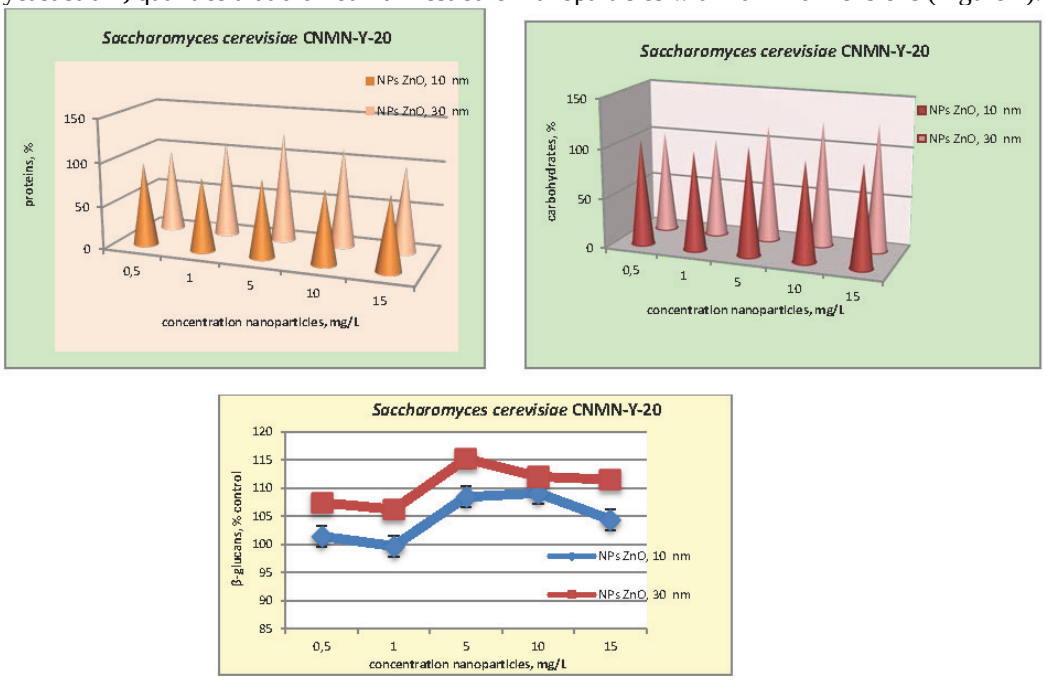

Figure 4. Content of proteins, carbohydrates and β-glucans in *Saccharomyces cerevisiae* CNMN-Y-20 biomass at the cultivation in presence of ZnO nanoparticles with different dimensions.

The similar effects were obtained in the case of study of influence of Fe_3O_4 nanoparticles on biosynthesis pigmented yeast strain *Rhodotorula gracilis* CNMN-Y-30. The research revealed that nanoparticles with 30 nm dimension possessed benefic effects on biosynthesis of cellular components, compared to other nanoparticles of 10 nm that inhibited the biosythesis of proteins, carbohydrates, carotenoids, the last being a part of category of micromolecular antioxidants that protect cells against free radical damage (Figure 5).

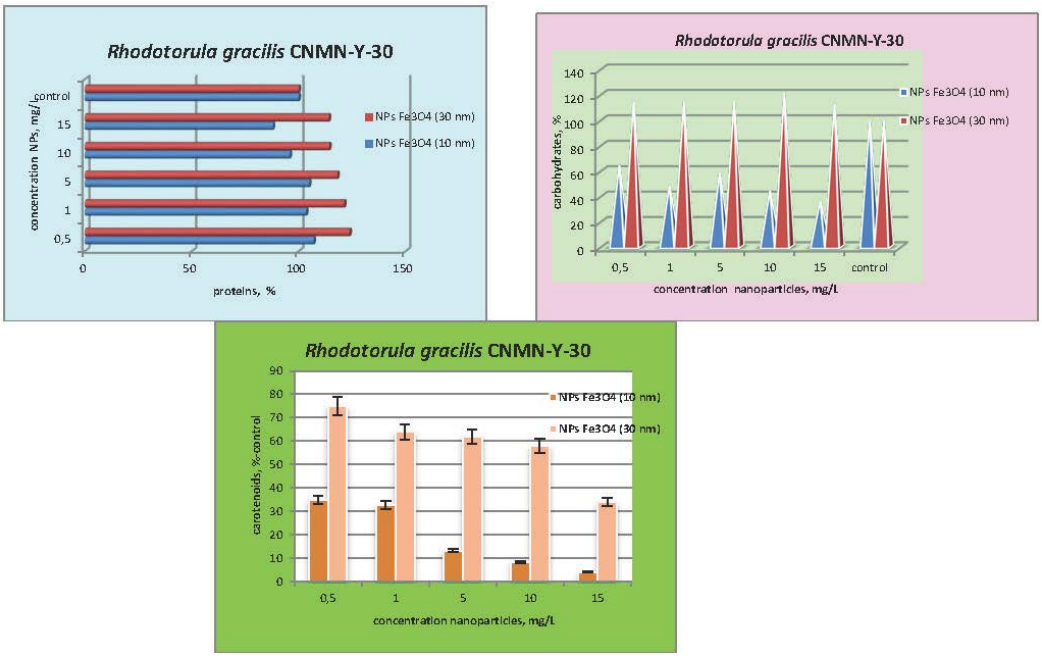

Figure 5. Proteins, carbohydrates, carotenoids pigments content in the *Rhodotorula gracilis* CNMN-Y-30 biomass at the cultivation in presence of Fe_3O_4 nanoparticles with different dimensions.

Thus, the determination of bioactive principles content modifications at *Saccharomyces* and *Rhodotorula* yeasts under the influence of ZnO and Fe_3O_4 nanoparticles has revealed various cell response induced by nanoparticles action depending on dimension. Nanoparticles with the dimensions under 30 nm used at the yeasts cultivation were more toxic as compared with those with larger dimensions. These effects were established by the determination of proteins, carbohydrates, carotenoids pigments content.

CONCLUSIONS

Thus, summarizing obtained results, it can be affirmed that new information regarding the peculiarities of action of TiO_2, ZnO, Fe_3O_4 nanoparticles on *Saccharomyces* and *Rhodotorula* yeast strains. TiO_2, ZnO, Fe_3O_4 nanoparticles induced alteration in synthesis of cellular components of studied yeast strains. Major factors determining cell response to the action of metallic nanoparticles are concentration and dimension. The important indices of this adaptive reaction are proteins, carbohydrates, including β-glucans and mannoproteins, and carotenoids pigments content.

The results obtained at the establishment of the characteristics of influence of TiO_2, ZnO, Fe_3O_4 nanoparticles on biosynthetic processes at *Saccharomyces cerevisiae* CNMN-Y-18, *Saccharomyces cerevisiae* CNMN-Y-20 and *Rhodotorula gracilis* CNMN-Y-30 can serve as a platform for following investigations to modulate biosynthesis of bioactive substances of biotechnological interest.

The research has important implications both from theoretical aspect for the explanation or confirmation of some fundamental hypotheses of the influence of nanoparticles on yeasts, and from practical aspect for the elaboration of some procedures for obtaining of bioactive principles of great interest.

REFERENCES

1. **Aguilar-Uscanga, B., Francois, J.M.,** (2003): *A study of the yeast cell wall composition and structure in response to growth conditions and mode of cultivation*. Letters in Applied Microbiology, 37: 268-274.
2. **Chiselița, O., Usatîi, A., Taran, N., Rudic, V., Chiselița, N., Adajuc, V**. ,(2010): *Tulpină de drojdie Saccharomyces cerevisiae – sursă de β-glucani*. Brevet de invenţie MD 4048. MD-BOPI, 6/2010.
3. **Dey, P., Harborne, J.,** (1993): *Methods in Plant Biochemistry. Carbohydr. Academic Press*, v. *2*, 529 p.
4. **El-Banna, A. A., Amal, M. A. El-Razek, A. R., El-Mahdy,** (2012): *Some Factors Affecting the Production of Carotenoids by Rhodotorula glutinis var. glutinis.* Food and Nutrition Sciences, 3: 64-71.
5. **El-Said Karim Samy, Ehab Mostafa Ali, Koki Kanehira, Akiyoshi Taniguchi**, (2014): *Molecular mechanism of DNA damage induced by titanium dioxide nanoparticles in toll-like receptor 3 or 4 expressing human hepatocarcinoma cell lines.* Journal of Nanobiotechnology, 12:48.
6. **Espita, P., Soares, N., Coimbra, J., Nélio, J., Cruz,R., Medeiros, E.,** (2012): *Zinc Oxide Nanoparticles: Synthesis, Antimicrobial Activity and Food Packaging Applications.* Food Bioprocess Technol. 5:1447–1464 DOI 10.1007/s11947-012-0797-6
7. **Frengova, G., Simova, E., Grigorova, D**., (1994): *Formation of carotenoids by Rhodotorula glutinis in whey ultra filtrate.* Biotechnology and Bioeng., 44: 8, 288-294
8. **Gardikiotis, F**., (2012): *Utilizarea fluidelor de nanoparticule în tratamentul afecţiunilor oftalmologice***.** Rezumatul Tezei de Doctorat. Iaşi, 54 p.
9. **Guiden D., Vinayak D., Ming Su, Lei Fu.** (2004): *Smart Therapy: The Multivariate Potentials of Iron Oxide Nanoparticles in Drug Delivery.* Nanoscape, 1, 71-75.
10. **Gutul, T., Rusu, E., Condur, N., Ursaki, V., Goncearenco, E., Vlazan, P.,** (2014): *Preparation of poly(N-vinylpyrrolidone)-stabilized ZnO colloid nanoparticles*. Beilstein J. Nanotechnol., 5: 402–406. doi:10.3762/bjnano.5.47.
11. **Hongtao Cui , Yan Liu, Wanzhong Ren,** (2013): *Structure switch between α-Fe_2O_3, γ-Fe_2O_3 and Fe_3O_4 during the large scale and low temperature sol–gel synthesis of nearly monodispersed iron oxide nanoparticles*. Advanced Powder Technology, 24, 1, 93–97.
12. **Kiran, G., Lipton, A., Sethu, P., Kumar, A., Joseph, S**., (2014)*: Effect of Fe nanoparticles on growth and glycolipid biosurfactant production under solid state culture by marine Nocardiopsis sp. MSA13A.* BMC Biotechnology, 14:48.
13. **Liu Hong-Zhi, Qiang Wang, Yuan-Yuan Liu, Fang Fang**., (2009): *Statistical optimization of culture media and conditions for production of mannan by S. cerevisiae.* Biotechnology and Bioprocess Engineering, 14: 577-583 DOI/10.1007/s12257-008-0248-4
14. **Lowry, O., Rosebough N., Farr A.** (1951): *Protein measurment with the folin phenol reagent*. J. Biol. Chem. 193: 265-275.
15. **Minju J., Park J.M., Lee E.J., Cho Y.S., Lee Ch., Kim J.M., Hah S.S.,** (2013): *Cytotoxicity of Ultra-pure TiO_2 and ZnO Nanoparticles Generated by Laser Ablation.* Bull. Korean Chem. Soc., 34, 11, 3301-3306.
16. **Mrinmoy De Partha, S. Ghosh, and Vincent M. Rotello.** (2008): *Applications of Nanoparticles in Biology*. Adv. Mater., 20: 4225–4241 DOI: 10.1002/adma.200703183.
17. **Nasr, N. F**. (2015): *Applications of Nanotechnology in Food Microbiology*. Int. J. Curr. Microbiol. App. Sci., 4(4): 846-853.
18. **Otero-Gonzalez Lila, Citlali Garcia-Saucedo, James A. Field, Reyes Sierra-Alvarez.** (2013): *Toxicity of TiO2, ZrO2, Fe0, Fe2O3, and Mn2O3 nanoparticles to the yeast, Saccharomyces cerevisiae*. Chemosphere, 93: 1201–1206.
19. **Pişkin S., Palantöken A., Yılmaz M.** (2013): *Antimicrobial Activity of Synthesized TiO_2 Nanoparticles.* International Conference on Emerging Trends in Engineering and Technology (ICETET'2013) Dec.7-8, 2013. PatongBeach, Phuket (Thailand).http://dx.doi.org/10.15242/IIE.E1213004.
20. **Potara Monica.** (2012). Obtinerea de noi nanoparticule plasmonice invelite in chitosan pentru detectie biomoleculara si activitate antibacteriana. Rezumatul Tezei Doctorale, CLUJ-NAPOCA, 33 p.
21. **Rai Mahendra, Nelson Duran,** (2011): *Metal Nanoparticles in Microbiology*, 305 p. ISBN 978-3-642-18311-9 e-ISBN 978-3-642-18312-6 DOI 10.1007/978-3-642-18312-6 Springer Heidelberg Dordrecht London New York.

22. **Sahayaraj K., Rajesh S.,** (2011): *Bionanoparticles: synthesis and antimicrobial applications. Science against microbial pathogens: communicating current research and technological advances.* A. Méndez Vilas (Ed.) FORMATEX, 228-244.
23. **Tămaș, V., Neamțu G.,** (1986): *Pigmenți carotenoidici și metaboliți. Chimie și biochimie*. București, v. 1, 269 p.
24. **Thammakiti, S., Suphantharika, M., Phaesuwan, T., Verduyn**, (2004): *Preparation of spent brewer's yeast β-glucans for potential applications in the food industry*. International Journal of Food Science&Technology, 39(1), 21-29.
25. **Usatîi, A., Molodoi, E., Efremova, N., Chiselița, N., Borisova, T., Fulga, L**., (2013): *Tulpină de drojdii Saccharomyces cerevisiae – producătoare de manani.* Brevet de invenție 4216 MD, BOPI nr. 4/2013, 24.
26. **Vaseem Mohammad, Ahmad Umar, Yoon-Bong Hahn**, (2010): *ZnO Nanoparticles: Growth, Properties, and Applications*. *Metal Oxide Nanostructures and Their Applications.* Chapter 4. ISBN: 1-58883-170-1Copyright © 2010 by American Scientific Publishers All rights of reproduction in any form reserved. Edited by Ahmad Umar and Yoon-Bong Hahn Volume 5: Pages 1–36
27. **Zhang Y.T., Gu W.Y.** (1999): *Determination of Mannose in Yeast by Ultraviolet Spectrometry*. Food. Ferment. Ind., 5, 32-36.

Acknowledgements.
The research was conducted within the project 15.817.05.16 A, funded by CSSDT of ASM, Moldova.

1 - Institute of Microbiology and Biotechnology of Academy of Sciences of Moldova, Chisinau, MD-2028, tel. +373(22)73-80-13
***** - usatyi.agafia@gmail.com