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CAROTENOID BIOSYNTHESIS POTENTIAL AND MORPHOLOGICAL CHARACTERS OF *Rhodotorula gracilis* YEAST UNDER THE ACTION OF Fe_3O_4 NANOPARTICLES

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This paper presents the results of the influence of Fe_3O_4 nanoparticles with dimensions of 10 nm in different concentrations on the biosynthesis potential of carotenoid pigments and morphological characters of strain of *Rhodotorula gracilis* CNMN-Y-30 yeast strain. Fe_3O_4 (10 nm) nanoparticles in concentrations of 0.5 to 15 mg/L have been found to induce significant changes in carotenoid formation and morphological carbohydrate formation in the yeast strain expressed by decreasing the amount of carotenoids in cell biomass and cell sizes. The correlation between the amount of carotenoids and the cell range is strong. The results obtained can contribute to further research in order to develop the theoretical and practical bases for the use of Fe_3O_4 nanoparticles in nanobiotechnologies.

Keywords: *Rhodotorula gracilis, Rhodosporidium toruloides, nanoparticles Fe_3O_4, carotenoids, morphological characters.*

POTENȚIALUL DE BIOSINTEZĂ AL CAROTENOIDELOR ȘI CARACTERELE MORFOLOGICE ALE LEVURII *Rhodotorula gracilis* SUB ACȚIUNEA NANOPARTICULELOR Fe_3O_4

În lucrare sunt prezentate rezultatele influenței nanoparticulelor Fe_3O_4 cu dimensiuni de 10 nm, în diferite concentrații, asupra potențialului de biosinteză al pigmenților carotenoizi și caracterelor morfologice ale tulpinii de levuri *Rhodotorula gracilis* CNMN-Y-30. S-a constatat că nanoparticulele Fe_3O_4 (10 nm) în concentrații de la 0,5 până la 15 mg/L induc modificări semnificative ale capacității de formare a carotenoidelor și caracterelor morfologice la tulpina de levuri, exprimate prin micșorarea cantității de carotenoide în biomasa celulară și a dimensiunilor celulelor. Raportul corelațional dintre cantitatea de carotenoide și aria celulelor este unul puternic. Rezultatele obținute pot contribui la cercetările ulterioare în scopul dezvoltării bazelor teoretice și practice de utilizare a nanoparticulelor Fe_3O_4 în nanobiotehnologii.

Cuvinte-cheie: *Rhodotorula gracilis, Rhodosporidium toruloides, nanoparticule Fe_3O_4, carotenoizi, caractere morfologice.*

Introduction

Nanoparticles (NPs) are a wide class of materials that have dimensions 1-100 nm and present practical interest for various fields such as electronics, medicine, pharmaceutical industry, food industry and cosmetic industries [5,9,15,20,23]. There is a number of risks associated with the production and use of metal oxide nanoparticles [14,19]. Recent studies have demonstrated that nanoparticles due to their smaller size than cells and cell organelles, are very mobile and penetrate biological structures, thus disrupting their normal functioning [6,22].

A major importance for the determination of the potential effects of nanoparticles on living organisms present research with the application of biological experimental models. The potential risks and safety of nanoparticles can be evaluated using *Rhodosporidium (Rhodotorula*) pigmented yeasts as study objects. In recent publications, the synonym of *Rhodotorula gracilis*, according to taxonomy, is the scientific name *Rhodosporidium toruloides*. Of particular interest today is the use and testing of metal nanoparticles, especially Fe_3O_4. Iron oxide nanoparticles possess unique physico-chemical, optoelectronic and biological properties. The increased number of commercial products, from cosmetics to pharmaceuticals obtained with the application of nanomaterials could be accidentally released into the environment. The recent publications demonstrates that nanoparticles of iron oxide have a practical interest in various fields, including microbial biotechnologies in order to obtain valuable bio-products [10,13,18]. It is also known that iron oxide is an extremely reactive oxidant. The cytotoxicity of reactive oxygen species is strongly mediated by iron ions. Disturbances induced by the action of iron ions may cause toxic reactions that result in the production of peroxides and free radicals, which affect cellular components, lipids, proteins, and carbohydrates.

In this context, investigations referring to the determination of character of modifications induced by nanoparticles of iron oxide on content of carotenoid pigments in correlation with the morphological changes

of the pigmented yeasts are opportune. The obtained results could be used for biotechnological valorification of biosynthetic potential of yeasts.

Materials and methods

Objects for study. In the research was used pigmented yeast *Rhodotorula gracilis* - CNMN-Y-30, selected as the manufacturer of protein and carotenoids. The strain is preserved in the collection of Yeasts Technology Laboratory and in the Collection of Nonpathogenic Microorganisms of within Institute of Microbiology and Biotechnology of Academy of Sciences of Moldova.

Nanomaterials. In the investigations Fe_3O_4 nanoparticles (10 nm) the suspension was prepared according to the method specified [17] made available for us with great kindness by researchers of the Institute of Electronic Engineering and Nanotechnologies "D.Ghițu" of the Academy of Science of Moldova. The concentrations of nanoparticles used in experiments to cultivate yeasts constituted 0.5; 1.0; 5.0; 10 to 15 mg/L. The variant without application of nanoparticles was used as the control sample.

Culture Media. For inoculation and submerged cultivation of yeasts there was used the fermentation media specific to strains in YPD study and wort malt [1,2]. 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.

Methods of achieving research. *The morphological* characters of the levurian culture were determined according to the identification indices stipulated in the papers [3,4]. The shape and size of the cells were examined in cultures on the YPD nutrient medium recommended for yeasts. After sowing, the stalks were incubated at 25-28° C. After 6, 24 and 120 hours of cultivation, yeasts were prepared from the culture (fixed cells). Fixed cell smears were stained using the gentian violet solution [12]. With the help of the XSZ-500 microscope, 100x / 1.25 OIL, 160 / 0.17 and MEM1300 camcorder, using the Future WinJoe special program, the cell shape, budding mode, cell sizes were determined. Colonial morphological characters were established according to the principles and techniques of general microbiology [27]. Macromorphological assessments were made by sowing yeast cultures on malt solid malt using the starch method (exhausted stalk). Incubation was carried out at 28° C for 5 days. The morphological assessment of the colonies was achieved by noting the shape of the colonies, the size, the profile, the gloss, the transparency, the color, the edge of the colony, the consistency. Carotenoids pigments were extracted from yeast biomass and measured spectrophotometrically to the method [21]. The degree of correlation between the level of morphological characters and carotenoid content was determined using Excel instruments, applying the r2 = r2 xy determination coefficient. Statistical processing of results was done using statistical software kit 7 veracity compared to the control $p \leq 0.05$.

Results and discussion

An important indication for evaluating the effect of nanoparticles on pigmented yeasts is the content of carotenoid pigments. Carotenoids are an ubiquitous group of isoprenoid pigments made up of 40 biosynthesis-derived biosyntheses of two generations of geranyl-transferase pyrophosphate and are non-polar solvents [11,24]. Because of their structure, carotenoid pigments act as antioxidants that protect the membrane, capture O_2 and peroxyl radicals. They are natural colors, yellow to red, so they have a great influence on the acceptability of many foods. Furthermore, some carotenoids are precursors of vitamin A; from the point of view of human health, these are among the well-respected biochemical factors that reduce the risks for degenerative diseases such as cancer, cardiovascular disease, macular degeneration and cataracts [13]. Carotenoids appear in the photosynthetic systems of the superior plants, algae and phototrophic bacteria. On the other hand, in non-photosynthetic organisms, carotenoids are important in protecting against photo-oxidative damage. Thus, many non-phototrophic bacteria and fungi are based on carotenoids for protection when they grow in conditions where light and air are abundant [7,16]. In the experiments were studied the changes induced by nanoparticles of Fe_3O_4 magnetite (10 nm) on the biosynthesis potential of carotenoid pigments and morphological characters at pigmented *Rodotorula gracilis* CNMN-Y-30, with superior biotechnological qualities. The results obtained showed that with the increase of Fe_3O_4 nanoparticles concentrations from 0.5 to 15 mg / L there was a significant decrease of β-carotene, toruline, torulorodine content in the yeast biomass in all experimental samples, which means that the processes biosynthesis are severely affected. Under the influence of nanoparticles at a concentration of 15 mg / L, the amount of pigments in levurian biomass decreased compared to the 74.5% (β-carotene), 77.1% (toruline) and 73.2% (torularodine) (Fig.1).

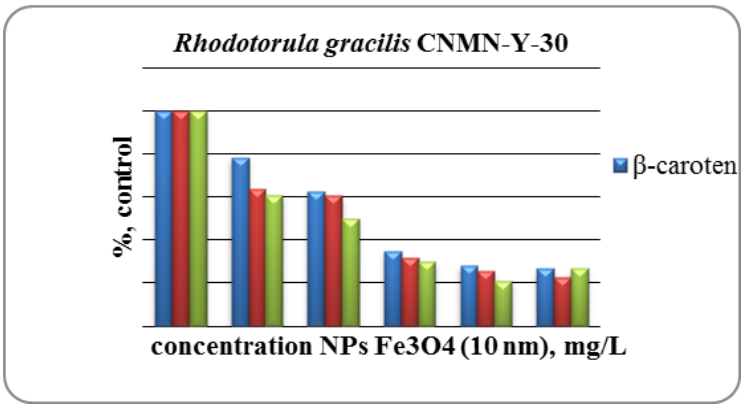

Fig.1. The influence of Fe_3O_4 (10 nm) nanoparticles on carotenoids content in biomass *Rhodotorula gracilis* CNMN-Y-30, contact time 120 hours.

Analyzing the data obtained in the evaluation of the influence of the nanoparticles of the magnet on the morphological characters of the yeast strain we found some differences in the effects produced by the nanoparticles. The results of the study by the microscopy method were confirmed and supplemented with images of the morphology of *Rhodotorula gracilis* CNMN-Y-30 cells, caused by the action of Fe_3O_4 nanoparticles, contact time 120 hours. According to the observations (fig. 2), submerged cells on the YPD medium showed elongated or globular, isolated or pairs of varying sizes. A category of alterations that can be observed in yeast cultures in cultivation in the presence of Fe_3O_4 nanoparticles is partial or total overflow of cellular content due to damage to the wall and cell membrane. Figure 2 (10 and 15 mg/L) shows fragments of cells lacking total or partial content.

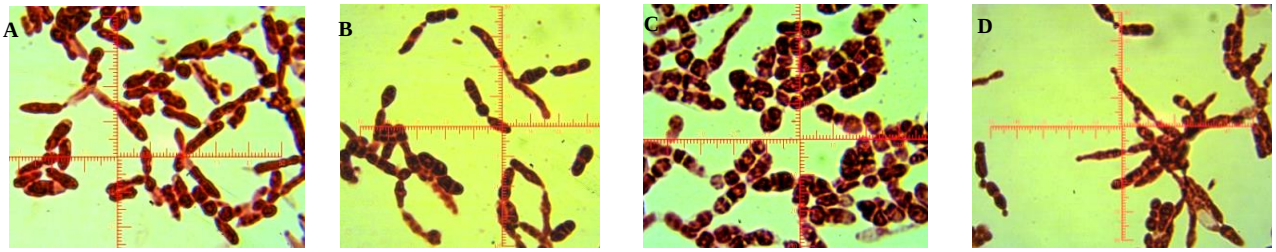

Fig.2. Morphological changes of *Rhodotorula gracilis* CNMN-Y-30 cells, caused by the action of Fe_3O_4 nanoparticles, contact time 120 hours, where: A – Control; B – 5 mg/L; C – 10 mg/L; D – 15 mg/L (ocular 100x/1.25 OIL, 160/0.17).

Examination of cell sizes led to the conclusion that the control culture exhibits typical cells with cell length dimensions ranging from 4-17.6 (mean 8.07) microns and 3-5 μm (3.4 microns). The mean cell range in the control variant is 21.53 micrometer squares (Table 1). The action of Fe_3O_4 nanoparticles (10 nm) is confirmed by changes in cell dimensions and agglomeration, especially the effect is more evident in 15 mg / L cell contact. In experimental variants, the cell lengths vary on average from 5.1 to 6.01 micrometers and the mean width from 3.29 to 4.43 micrometers. The average area of the cells decreases and is within the range of 14.43-18.68 microns, which is 67-86% relative to the control (Table 1).

Table 1

Average sizes of strains of *Rhodotorula gracilis* strain CNMN-Y-30, cultivated for 120 hours in the presence of Fe_3O_4 nanoparticles

Concentration of Fe_3O_4 nanoparticles	Cells examined, (n)	Average length (D), μm	Average width (d), μm	Cell area, (A=π/4xDd)	
				μm²	% to control
Control	26	8,07±3,49	3,4±0,61	21,53	100
5 mg/L	33	6,01±2,74	3,96±0,87	18,68	86
10 mg/L	34	5,25±1,42	4,32±1,01	17,8	82,6
15 mg/L	36	5,44±2,37	3,31±0,9	14,13	65,6

In order to extend the research to ensure the credibility of the results obtained on the influence of the nanoparticles on the morphological characters of the yew culture, morphological assays of the colonies were performed. To obtain the colonies, samples of control and experimental yeast suspensions, with contact duration of 120 hours nanoparticles, were seeded on solidified malted wort media using the method in stria (exhausted anneal) (Fig.3).

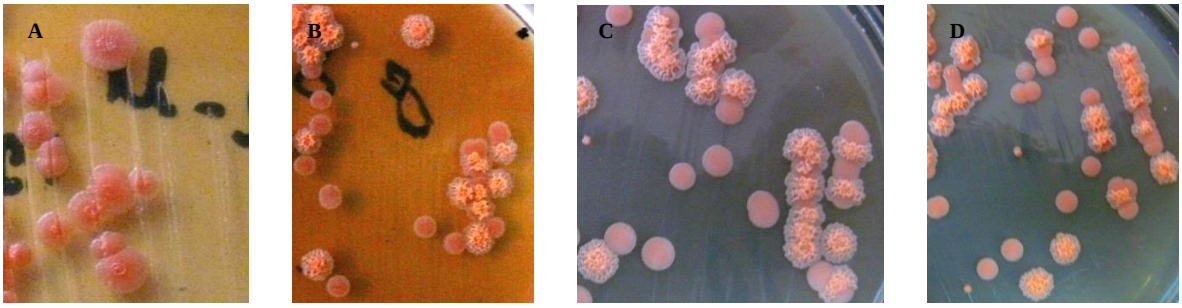

Fig.3. Morphological alterations of *Rhodotorula gracilis* CNMN-Y-30 colonies, caused by the action of Fe_3O_4 nanoparticles, contact time 120 hours, where: A – Control; B – 5 mg/L; C – 10 mg/L; D – 15 mg/L.

Regarding the control culture, we mention 5-12 mm colonies formed on solidified malt wort media, the shape of the circular colonies, the edges of the slightly wavy colony, the surface of the wrinkled colony with the nipple center, the intensely coral-orange color. Examining the morphological features of culture colonies in contact with Fe_3O_4 nanoparticles, we observe some changes in the shape and profile of colonies, color and size. As a result of analyzes of the appearance and morphology of the colonies, some peculiarities were observed which can be classified into two groups: in the first group (specific for all nanoparticle concentrations) - colonies 4-10 mm in size, margins of slightly corrugated colony, with nipple center, coral color - pale orange; of the second group (specific characters for the concentrations of 10 and 15 mg / L nanoparticles) mention colonies of 2-5 mm, round, smooth, glossy, coral-orange color.

In order to elucidate the links between the carotenoid biosynthesis potential and the morphological characteristics of yeast culture under the action of nanoparticles, the correlation ratio was calculated. Thus, it has been found that with the increase in the concentration of nanoparticles in the YPD medium, both the cellular area and the amount of synthesized carotenoids are decreased. The coefficient of determination between the cell area and the amount of carotenoids at *Rhodotorula gracilis* CNMN-Y-30, at cultivation in the presence of Fe_3O_4 nanoparticles, 120 hours contact time, is 0.7494 or 74% and confirms the existence of a true dependence between these two parameters.

Conclusions

The paper presents the results of the study of the influence of Fe_3O_4 nanoparticles (10 nm) on *Rhodotorula gracilis* CNMN-Y-30 pigmented yeast strain. The study of Fe_3O_4 nanoparticles (10 nm) in concentrations from 0.5 to 15 mg / L demonstrated changes in carotenoid formation capacity and morphological characteristics of *Rhodotorula gracilis* CNMN-Y-30 yeast culture. Significant changes are noted by diminishing stem cell sizes, which correlate with decreased carotenoid pigment formation capacity in cell biomass. The correlation between the area of cells and the amount of carotenoids is a strong one $R^2 = 0.7494$.

The results indicate that the yeast culture pigmented remains vulnerable to iron oxide nanoparticles of 10 nm size. The results presented contribute to the development of the theoretical and practical bases for assessing the influence of nanoparticles on biological objects. The information obtained can be used by specialists in the field of microbiology, medicine, cosmetology, textile industry, food industry, etc., in which these nanomaterials have applications.

References:

1. AGUILAR-USCANGA, B., FRANCOIS, J. A study of the yeast cell wall composition and structure in response to growth conditions and mode of cultivation. In: *Letters in Applied Microbiology*, 2003, no 37, p.268-274.
2. ANGHEL, I., VAMANU, A., MITRACHE, L. Biologia şi tehnologia drojdiilor. In: *Tehnica Press.* Bucharest, 1993, p.308.

3. ANGHEL, I., VASSU, T., SEGAL, B., BERZESCU, P., HERLEA, V., DAN, V., CANCEA, I., VAMANU, K. Biologia şi tehnologia drojdiilor. In: *Tehnica Press*. Bucharest, 1991, p.315.
4. BARNETT, J., PAYNE, R., VARROW, D. Yeasts: characteristics and identification. 3-rd Edition. In: *Cambrige University Press*, 2000, p.1150.
5. BOISSEAU, P., LOUBATO, B. Nanomedicine, nanotechnology in medicine. In: *Comptes Rendus de L'Academie des Science*, 2011, p.7-18.
6. BRAIN, J., CURRAN, M., DONAGHEY, T., MOLINA, R. Biologic responses to nanomaterials depend on exposure, clearance, and material characteristics. In: *Nanotoxicology*, 2009, vol.3, p.174-180.
7. BRITTON, G, HORNERO-MÉNDEZ, D., Carotenoids and colour in fruit and vegetables. In: *Oxford Clarendon Press*, 1997, p.11-27.
8. DONG, M., WANG, X., HUANG, F., JIN, Z., LI, T. *Toxicity of Fe-0 Nanoparticles on the Denitrifying Bacteria-Alcaligenes Eutrophus. Materials for Environmental Protection and Energy Application*, 2012, p.343-344.
9. DONG, Y., LOVE, K., DORKIN, J., SIRIRUNGRUANG, S., ZHANG, Y., CHEN, D., ANDERSON, D. Lipopeptide nanoparticles for potent and selective si RNA delivery in rodents and nonhuman primates. In: *Proceedings of the National Academy of Sciences*, 2014, vol.111(11), p.3955-3960.
10. FARAJI, Y. Magnetic nanoparticles: synthesis, stabilization, functionalization, characterization, and applications. In: *Journal of the Iranian Chemical Society*, 2010, vol.7, p.1-37.
11. GOODWIN, T. Biosynthesis of carotenoids. In: *Methods in Enzymology*, 1993, vol.214, p.330-340.
12. GRAM, H. Gram staining. Fortschritte der Medicin. English translation *in:* Brock. *Milestones in Microbiology*, 1999, vol.2, p.215-218.
13. KAMAT, T., DEVASAGAYAM, K., PRIYADARSINI, I., MOHAN, P. Reactive oxygen species mediated membrane damage induced by fullerene derivatives and its possible biological implications. In: *Toxicology*, 2000, p.55-61.
14. KASEMETS, K., DUBOURGUIER, I., KAHRU, A. Toxicity of nanoparticles of ZnO, CuO and TiO_2 to yeast Saccharomyces cerevisiae. In: *Toxicology in Vitro*, 2009, vol.23, p.1116-1122.
15. LIU, H., LU, F., XING, R., ZHU, J. Structural effects of Fe_3O_4 nanocrystals on peroxidase-like activity. In: *Chemistry: A Eur J*, 2011, vol.17, p.620-625.
16. MERHAN, O. Biochemistry and Antioxidant Properties of Carotenoids. In: *Intech.*, 2017, p.51-66.
17. OTERO-GOZALIEZ, L., GARSIA-SAUCEDO, C., JAMEZ, A., SIERRA-ALVAREZ, R. Toxicity of TiO_2, ZrO_2, Fe^0, Fe_2O_3 and Mn_2O_3 nanoparticles to the yeast, Saccharomyces cerevisiae. In: *Chemosphere*, 2013, p.1201-1206.
18. RAI, M., DURAN, N. *Metal Nanoparticles in Microbiology.* 1rd Edition. Berlin: *Rai M & co.* 2011, p.305.
19. SASTRY, R., RASHMI, H., RAO, N. Nanotechnology for enhancing food security in India. In: *Food Policy*, 2011, vol.36, p.391-400.
20. SHAHWAN, T. Green synthesis of iron nanoparticles and their application as a fenton-like catalyst for the degradation of aqueous cationic and anionic dyes. In: *Chemical Engineering Journal*, 2011, vol.172, p.258-266.
21. TAMAS, V., NEAMŢU, G. *Carotenoids and metabolites*, 1986, vol.2, p.269.
22. Institutul de Microbiologie şi Biotehnologie al Academiei de Ştiinţe a Moldovei. *Procedeu de evaluare a toxicităţiii nanoparticulelor oxizilor de metale cu utilizarea levurilor*. Brevet MD nr.4522, Inventatori: USATÎI, A., BEŞLIU, A., CHISELIŢĂ, N., RUSU, E., GUŢUL, T, 2017, BOPI, 10.
23. WANG, C., CHENG, L., LIU, Z. Drug delivery with upconversion nanoparticles for multi-functional targeted cancer cell imaging and therapy. In: *Biomaterials*, 2011, 32(4), p.1110-1120.
24. WILLIAMS, A., BOILEAU, T., ZHOU, J., CLINTON, S., ERDMAN, J. β-Carotene modulates human prostate cancer cell growth and may undergo intracellular metabolism to retinol. In: *Journal of Nutrition*, 2000, p.728-732.
25. ZARNEA, G. Tratat de Microbiologie generală. In: *Romanian Academy Publishing House*, Bucharest, 1994, p.169-170.

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