BIOSYNTHESIS OF β-GLUCANS AND MORPHOLOGICAL FEATURES *SACCHAROMYCES CEREVISIAE* CNMN-Y-20 YEASTS UNDER THE ACTION OF ZINC OXIDE NANOPARTICLES

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Abstract: The paper provides new information on β-glucans biosynthesis capacity and the morphological features modification of cells and colonies of *Saccharomyces cerevisiae* CNMN-Y-20 yeast strain known as producer of β-glucans. It was found that the development cycle and bioproductive capacity of the yeast was affected by cultivation in the presence of ZnO in a concentrations and the contact duration manner. Within 6-24 hours, the reproduction of the nanomodified cell was decreased compared to the control, but after 120 hours of submerged cultivation an insignificant increase in biomass content relative to 1L culture medium was recorded predominantly related to nanoparticle concentrations 0.5-1.0 mg/L. ZnO nanoparticles (<100 nm). Concentrations of 0.5-15 mg/L initiated a 13-15% increase in average dimensions of *Saccharomyces cerevisiae* CNMN-Y-20 cells and 7-12% increase in the ability to form of β-glucans, especially at concentrations of 1 and 5 mg/L. The correlation coefficient between the cells area and the β-glucans amount is strong (R^2 = 0.8021). The results provide the possibility of enhancing of the range of analyzes and the formation of reference bases necessary for the strategy to enlarge the biotechnological performance of yeasts.

INTRODUCTION

Glucan is a complex polysaccharide present in the yeast cell wall, composed of β-(1→6) and β-(1→3) linked D-glucopyranose units (Lesage et al., 2006). The β-glucans extracted from the yeast cell walls possess high biological activity, in particular, immunomodulatory, anticancer, antimicrobial (Hunter et al., 2002; Volman et al., 2008; Yoon et al., 2008; Pillai et al., 2005; Thammakiti et al., 2007) and are widely used at production of medical preparations, as active part of cosmetic remedies and in other fields. β-glucans obtained from yeasts were approved by the European Food Safety Authority as new food ingredients (EFSA, 2011). The genus *Saccharomyces* presents a great deal of scientific and practical interest among other β-glucans producing yeasts (Cabib et al., 2012; Novak et al., 2012).

The analysis of fields of β-glucans utilization of microbial origin demonstrates the importance of searching for new ways of producing this polysaccharide component. Currently, more research has been done on the use of metal oxides nanoparticles in the biotechnology of microorganisms (Rai, Duran, 2011). Nanotechnologies have the potential to influence different areas, including the food, pharmaceutical and cosmetic industries, and microbial biotechnology (Mrinmoy De Patha et al., 2008). According to some authors, the application of nanoparticles in the biotechnology of microorganisms cultivation ensures increased absorption of the necessary nutrients and may change the metabolic processes (Ban, et al., 2014).

A new approach for increasing the β-glucans production potential would be the application of metal oxides nanoparticles in the biotechnology processes of yeast cultivation. The search for nanoparticles suitable for yeasts frowth and development is of major importance. Among metal oxides nanoparticles, preferential are zinc oxide nanoparticles. The perspectives of applying ZnO nanoparticles in different fields are mentioned in several researchers publications (Espita et al., 2012; El-Diasty Eman et al., 2013). The microbial metabolic complexity complicates the analysis and identification of the nanoparticle-cell interaction. According to some investigations, the mechanism of ZnO nanoparticles influence on microbial cell is complex and induces changes in both cell membrane and cytoplasm (Ya-Nan Chang et al., 2012).

In order to develop the technological possibilities for production of new bioproducts, including β-glucans with polyvalent properties, it is important to investigate in detail the impact caused by nanoparticles on the development and production of metabolites by yeasts with broad potential in biotechnological applications.

Thus, the present paper discusses the results of the elucidation of β-glucans biosynthesis potential and changes in cellular and colonial morpho-cultural characteristics of *Saccharomyces cerevisiae* CNMN-Y-20 yeast strain under the action of zinc oxide nanoparticles.

MATERIALS AND METHODS

Strains, culture medium, cultivation conditions. The yeast strain *Saccharomyces cerevisiae* CNMN-Y-20 was used as a model organism for β-glucans producer (Chiseliţa et al., 2010). 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.

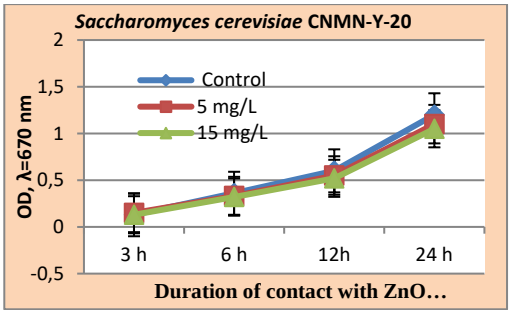
The YPD fermentation medium (Aguilar-Uscanga et al., 2003) specific for selected yeast strains was used for inoculation and submerged cultivation of yeasts. The submerged cultivation was carried out in depth capacity 1 liter Erlenmeyer flask, shaker (200 rpm) at temperature of 25ºC, aeration rate 80.0-83.0 mg/L, for 120 hours. Yeast cells at a concentration of $2x10^6$ cells/ml were inoculated on the liquid medium at 5% ratio.

Zinc oxide nanoparticles (ZnO Np) nanopowder, <100 nm particle dimension were from SIGMA-ALDRICH. The suspension was prepared according to the method specified (Oterro-Gonzalez et al., 2013). The final concentrations of nanoparticles used at the yeast cultivation were 0.5; 1.0; 5.0; 10 and 15 mg/L. The variant without application of nanoparticles served as control sample.

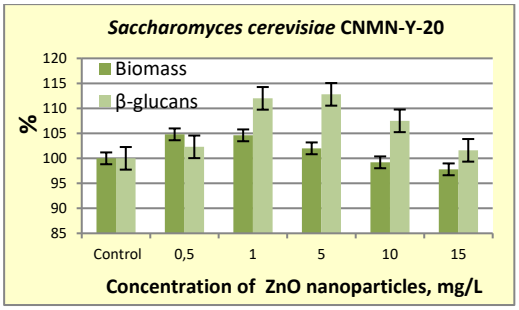
Methods. The total number of cell obtained at liquid medium was determined spectrofotometrically according to the known methods (Mitcell et al., 2004; Dobias, 2013). Yeasts biomass was determined gravimetrically (Hong-Zhi et al., 2009). The β-glucans content in the yeast biomass was determined gravimetrically as described (Thammakiti et al., 2004). Morphological characters of yeast strain were established according to indices described in (Barnet, et al., 2000; Kreeger-Van Rij, 1984; Anghel et al., 1991). The shape and dimension of the cells was examined at YPD nutritive medium, recommended for yeasts. After inoculation, samples were incubated at 25-28°C. Preparation of yeast cells by tehnique of fixation was effectuated after 6, 24 and 120 hours of cultivation. Fixed yeast smears were stained using the gentian violet solution staining technique. The cell shape, budding mode, cell dimensions were determined using XSZ-500 microscope, 100x/1.25 OIL, 160/0.17 and MEM1300 video camera and Future WinJoe special program. Colony morphology was established according to the principles and techniques of general microbiology (Zarnea et al., 1992). Macromorphological investigations were made by inoculation of yeast cultures on solid beer wort using the inoculation loop. 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. The degree of correlation between morphological characters and β-glucans content was established using Microsoft Excel. Statistical processing of results was carried out using statistical software kit 7. Statistical processing of obtained results was effectuated electronically with the calculation of the standard errors for the relative and average values, the differences between the experimental and control data were established using Student's t-test and P value.

RESULTS AND DISCUSSIONS

The results of the analysis of cell multiplication dynamics, biomass and β-glucans production by *Saccharomyces cerevisiae* CNMN-Y-20, under the influence ZnO nanoparticles (<100 nm), revealed new data on the effects of nanoparticles produced according to concentration and duration of contact with yeasts. As a result of this investigation, we showed that cells of the control sample and those cultivated in the presence of nanoparticles had the identical *lag* phase (Figure 1). Over the period of 6-24 hours, the process of cell multiplication is declining in relation to the control. In the case of utilization of 5 and 10 mg/L nanoparticles, a decrease in optical density was observed, the difference from the control after 24 hours being 8.3-12.5%.



a)



b)

Figure 1. The dynamics of cell multiplication (a), biomass content and β-glucans (b) at *Saccharomyces cerevisiae* CNMN-Y-20 under the ZnO nanoparticles action (<100 nm).

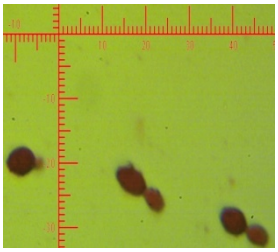
After the growth text, the biomass production after 120 hours of submerged cultivation on the YPD medium was evaluated. The biomass was collected by centrifugation and subjected to biochemical analysis. The presented figure 1b reveals data on the accumulated biomass content. In these experiments, there was an insignificant increase in biomass content, predominantly related to nanoparticle concentrations of 0.5-1.0 mg/L.

To a far greater extent, ZnO nanoparticles (<100 nm) have influenced the β-glucan biosynthesis process. An increase in the β-glucans content in samples cultivated in the presence of nanoparticles in concentrations of 1, 5 and 10 mg/L was recorded (Figure 1b). In these variants the β-glucans amount in the yeast biomass was increased by 7.5-12.8%.

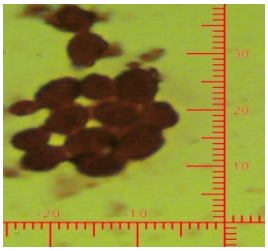
Generalizing research results, it can be mentioned that cell cycle and bioproductive parameters of *Saccharomyces cerevisiae* CNMN-Y-20 yeast strain in cultivation in the presence of ZnO nanoparticles (<100 nm) were modified depending on used concentrations and duration of contact. The increase of the rates of β-glucan accumulation in yeasts biomass was observed at the cultivation on the nutrient medium supplemented with ZnO nanoparticles in limits of concentrations of 1-5 mg/L.

Research that involved the analysis of morpho-cultural characters of cells and colonies caused by ZnO nanoparticles (<100 nm) has revealed different changes depending on applied concentrations. Thus, yeast cultures in the liquid medium both control and experimental samples produce cloudiness after 24 hours of incubation at 25°C forming a white foam, often a creamy ring is formed at the surface of the culture medium. After 72 hours of submerged cultivation, a compact sediment is formed, the yeast cells retain their flocculation ability.

After examination of *Saccharomyces cerevisiae* CNMN-Y-20 cells being in contact with ZnO nanoparticles (<100 nm) under a light microscope, it can be mentioned the lack of nanoparticle effects on the budding process of yeast. In both experimental and control variants, cells have demonstrated unipolar budding (Figure 2).



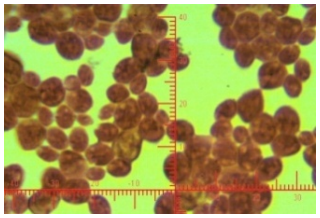
Control



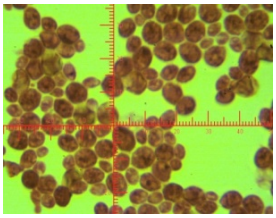
15 mg/L ZnO Np (<100 nm)

Figure 2. Budding *Saccharomyces cerevisiae* CNMN-Y-20 cells 6 hours of cultivation (100x/1,25 OIL, 160/0,17)

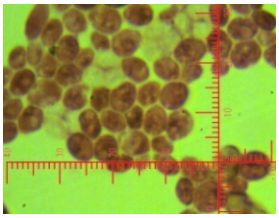
It should be noted that the average size of yeasts cells have changed depending on the applied concentrations of ZnO nanoparticles (<100 nm). After 120 hours of contact with ZnO nanoparticles (<100 nm), the cells cultivated on the YPD medium are generally elliptical, some being more elongated, some more rounded. The arrangement of the cells were in isolation, in pairs and in chains. Sometimes, after 120 hours of cultivation, partial or total cellular overflow occurs as a result of damage to cell membranes. The results obtained by the microscopy methods were confirmed by the images, which are presented below in Figure 3.



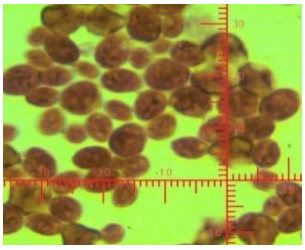
Control



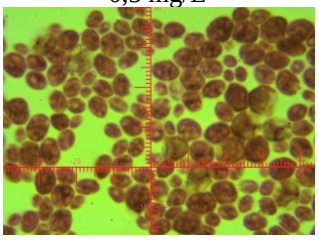
0,5 mg/L



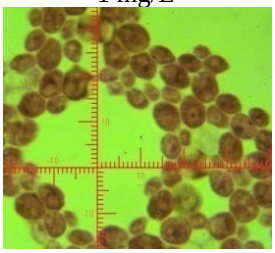
1 mg/L



5 mg/L



10 mg/L



15 mg/L

Figure 3. Morphological features of *Saccharomyces cerevisiae* CNMN-Y-20 cells, cultivated in the presence of ZnO nanoparticles (<100 nm), (contact time 120 hours), 100x/1.25 OIL 160/0.17

In a study effectuated to elucidate the specificity of the zinc oxide nanoparticles influence on the cellular parameters of yeast growth, the measurements of the cell length and width were performed, and the area of the cell was subsequently calculated. The mean values of the length, width and area of the examined yeast strain cells are presented in Table 1. From the table it is apparent that the average cell dimension range between 5.77-6.5 μm length and 4.92-

5.4 μm width. These cell characteristics, with some little deviations, are also seen in control samples. From the obtained data, the benefic influence of the concentrations of 1 mg/L and 5 mg/L is observed, the average area of the cells is slightly increased compared to the control and it is 26.28-26.63 μm^2, which is more with 13.8 and 15.4%.

Table 1 Average dimensions of yeast cells of Saccharomyces *cerevisiae* CNMN-Y-20 yeast strain, at the cultivation for 120 hours in presence of ZnO nanoparticles (<100 nm)

Nr	**Sample, ZnO NP** <100 nm	**Number of examinated cells**	**Average lenght (D), μm**	**Average width (d), μm**	**Cell area (A=π/4xDd)**	
					μm^2	**% of control**
1	Control	55	5.94±1.43	4.95±1.45	23.08	100
2	0.5 mg/L	50	6.24±1.67	5.02±1.68	24.58	106.4
3	1mg/L	44	6.50±1.35	5.22±1.53	26.63	115.4
4	5 mg/L	40	6.20±1.54	5.40±1.33	26.28	113.8
5	10 mg/L	63	6.05±1.53	5.11±1.68	24.26	105.1
6	15 mg/L	40	5.77±1.19	4.92±1.37	22.28	96.5

In order to determine the morphological features modification of *Saccharomyces cerevisiae* CNMN-Y-20 colonies, the both control and experimental variants of yeast culture were seeded on solid YPD medium. The results of the colony examinations are presented in Table 2. Research has demonstrated that ZnO nanoparticles (<100 nm) do not significantly modify the morphological features of *Saccharomyces cerevisiae* CNMN-Y-20 yeast strain. Thus, yeasts form on solid medium white or pale pink colonies, surface glossy, smooth, umbonate, the diameter varies between 3-8 mm, the characters observed for control and experimental variants. At the same time, in experimental samples with nanoparticles applied in concentrations 0.5 mg/L and 15 mg/L, colonies with special characters compared to the control were found. The differences were expressed by the undulate margins of the colonies and crateriform profile.

Table 2 Morphological features of *Saccharomyces cerevisiae* CNMN-Y-20 colonies, cultivated in the presence of ZnO nanoparticles (<100 nm), (contact time 120 hours)

ZnO NP <100 nm**)**	**Form of colonies**	**Profile of the yeast colonies**	**Transparency**	**Consistency**	**Colour**	**Dimension, mm**
Control	Circular, entire margins	Umbonate, glossy surface	opaque	slightly creamy	white or pale pink	3-5
0.5 mg/L	Circular, entire margins or undulate	Umbonate, or umbilical, glossy surface	opaque	slightly creamy	white or pale pink	3-8
1 mg/L	Circular, entire	Umbonate, glossy	opaque	slightly creamy	white or pale	3-5

	margins	surface			pink	
5 mg/L	Circular, entire margins	Umbonate, glossy surface	opaque	glutinous	white or pale pink	3-5
10 mg/L	Circular, entire margins	Umbonate, glossy surface	opaque	slightly creamy	white or pale pink	4-7
15 mg/L	Circular, entire margins or undulate	Umbonate, or umbilical, glossy surface	opaque	slightly creamy	white or pale pink	4-6

Morphological features of *Saccharomyces cerevisiae* CNMN-Y-20 colonies, cultivated in the presence of ZnO nanoparticles (<100 nm) were confirmed by the images presented below in Figure 4.

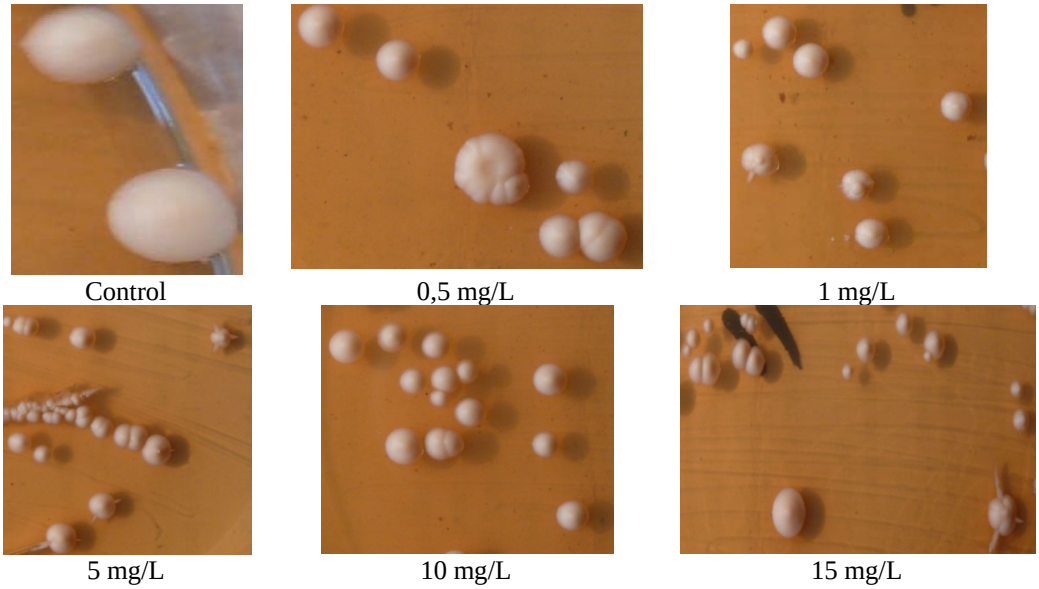


Figure 4. Morphology of *Saccharomyces cerevisiae* CNMN-Y-20 colonies at the cultivation in the presence of ZnO nanoparticles (<100 nm), contact duration 120 hours

In order to enhance the research and to establish the degree of correlation between morpho-cultural features and the bioactive principles production under the action of ZnO nanoparticles (<100 nm), the correlation between the average cell area and β-glucans content in yeast biomass has been determined. It was established that β-glucans content is concomitant ascending with average area of cell with the maximal effect at the nanoparticles concentrations of 1mg/L and 5 mg/L. In the samples where the cells were in contact with higher nanoparticles concentrations (10-15 mg/L), the dynamics of both tested parameters was decreased (Figure 5).

The correlation coefficient between the β-glucans amount and the average cell range is R^2 = 0.8021, which is considered to be a strong correlation.

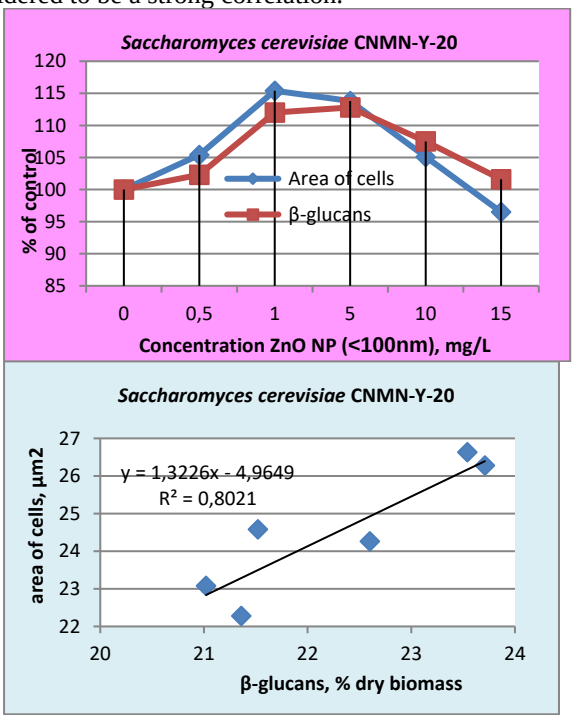


Figure 5. The correlation between the β-glucans content and cells area of *Saccharomyces cerevisiae* CNMN-Y-20 at ZnO nanoparticles (<100 nm) action, contact time 120 hours

Generalizing the latest information, it can be mentioned the importance of studying β-glucans due to their application in various fields such as medicine, food industry, pharmaceutical industry, etc. β-glucans can also be used as bioproducts with polifunctional properties, in particularly, immunomodulatory, anticancerigen and antimicrobial (Rondanelliet et al., 2009; Chan GC, 2009; Espita et al., 2012). The functionality of β-glucans and their production, to a large extent, depends on nutritive media composition and cultivation conditions. Specific literature reflects data on the use of metal oxides nanoparticles in biotechnology of cultivation of microorganisms (Vaseem et al., 2010; Dobias, 2013). In the present study, we analyzed the effects of ZnO nanoparticles (<100 nm) on *Saccharomyces cerevisiae* CNMN-Y-20 yeast strain in terms of determination of β-glucans formation capacity and morphological features modification. The effectuated experiences have been made elucidate effects in a different way.

CONCLUSIONS

We showed that the development cycle and the bioproductive parameters of the yeast cultivated in the presence of ZnO nanoparticles (<100 nm) changed depending on the used concentrations and the duration of contact. Within 6-24 hours, the reproduction of nanomodified

cell was decreased compared to the control, but after 120 hours of submerged cultivation there is an insignificant increase in biomass content relative to 1L culture medium, predominantly related to nanoparticle concentrations 0.5-1.0 mg/L.

ZnO nanoparticles (<100 nm), at concentrations of 0.5-15 mg/L initiated a 13-15% increase in average dimensions of *Saccharomyces cerevisiae* CNMN-Y-20 cells and 7-12% of the ability to form of β-glucans, especially at concentrations of 1 and 5 mg/L. The correlation coefficient between the area of cells and the β-glucans amount is strong.

Cellular morphology data (correlated to cell dimensions) correlated with those of the bioactive principles of biotechnology interest, β-glucans, may offer the possibility of enhancing of the range of analyses and the formation of reference bases necessary for the strategy to enlarge the biotechnological performance of yeasts in the case of metal oxides nanoparticles application.

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