THE EFFECT INDUCED BY MILLIMETER WAVES WITH THE FREQUENCY 53.33 GHZ ON *SACCHAROMYCES CEREVISIAE* CNMN-Y-18 YEAST STRAIN

AGAFIA USATÎI[1*], ELENA MOLODOI[1], NADEJDA EFREMOVA[1], LUDMILA FULGA[1]

Received: 5 November 2014 / Revised: 23 February 2015 / Accepted: 9 March 2015 / Published: 6 April 2015

Keywords: *Saccharomyces cerevisiae*, millimeter waves, mannoproteins, carbohydrates, cell biomass, protein, catalase activity.

Abstract: The effect of extremely high frequency electromagnetic waves on the biosynthetic activity of *Saccharomyces cerevisiae* CNMN-Y-18 yeast strain in dependence on the duration of irradiation was studied. The maximum amount of biomass, protein, carbohydrates, mannoproteins and catalase has been showed to accumulate when the yeast cells were irradiated with a frequency f = 53.33 GHz for 10 minutes. High degree of dependence between the content of cellular components (a correlation coefficient between $R^2 = 0.875$ and 0.926) it has been shown which demonstrates that biosynthetic processes were influenced by the same phenomenon - millimeter waves. A procedure for increasing of mannoprotein content in yeasts with the utilization of extremely high frequency waves has been proposed in this study.

INTRODUCTION

The concept of millimeter wave electromagnetic oscillations includes extremely high frequency (30-300GHz). Recent research regarding the effect of millimeter waves focused on experimental investigation at cellular level on millimeter wave's therapy application (Cifra et al., 2011; De Vita et al., 2011). Studies have demonstrated the influence of millimeter waves on the cell multiplication and proliferation, enzymes activity and other biological processes (Markkanen Ari, 2009). In the field of millimeter wave investigation enters and theoretical modeling of possible mechanisms of interaction with biological sentities, including microorganisms. An important characteristic of millimeter waves is specific resonant character. Millimeter wave oscillations superimposed cell vibrations can initiate positive or negative responses. The biological effect is usually observed within the small millimeter wave frequency interval (Ruiz-Gomez et al., 2004). According to previously study of the influence of millimeter waves with frequencies 60,12 GHz, 53,33 GHz şi 42,19 GHz on biosynthetic activity of *Saccharomyces cerevisiae* CNMN-Y-18, millimeter wave frequency 53.33 GHz has had maximum stimulating effect among other (Molodoi et al., 2014).Therefore, the establishing of mechanisms of cell reaction to the influence of millimeter waves is one of the important problems in ensuring the effectiveness of their practical application.

The aim of this investigation is to evaluate the influence of millimeter wave frequency f = 53.33 GHz on biosynthetic processes in yeasts in dependence on the duration of irradiation.

MATERIALS AND METHODS

Object study - *Saccharomyces cerevisiae* CNMN-Y-18 yeast strain, mannoproteins producer from National Collection of Nonpathogenic Microorganims of Institute of Microbiology and Biotechnology of Academy of Sciences of Moldova (Usatîi et al., 2013).

Media and cultivation conditions. Seed material obtained by growing yeast strain on the beer wort for 24 hours on shaker (200 rpm) was irradiated with millimeter wave frequency f = 53.33 GHz (corresponding to the wavelength $\lambda = 5.6$ mm) with the duration of treatment of 5, 10, 15, 20, 25 minutes. The irradiated inoculum in the amount of 5%, $2x10^6$ cells/ml concentration was transferred in nutritive medium YPD which consist of 1% yeast extract, 2% peptone, 2% glucose, water 1 L, pH 5.5 (Aguilar-Uscanga and Francois, 2003). Cell density was determined spectrophotometrically at 600 nm by UV-Vis spectroscopy. The submerged cultivation was carried out in Erlenmeyer flasks containing 0.2 L YPD, the concentration of oxygen - 81, 3..83,3 mg/l, cultivation period of 120 hours at temperature of 25˚ C.

As millimeter wave generator was used KWC-ND device, RS-232 (made in Russian Federation) with wavelength $\lambda =$ 4.9; 5,6; 7,1 mm,corresponding to the frequency *f*=60,12 GHz; 53,33 GHz; 42,19 GHz, kindly provided by the staff of the Institute of Electronic Engineering and Nanotechnologies ” D. Ghitu”. The device is certified and permitted for use in medical practice. The flask with 5 ml of inoculum was placed at the distance of 0,5 cm in relation to the generator.

Methods of investigation. Cell biomass was determined gravimetrically (Liu et al., 2009). Yeast total carbohydrates in the biomass were determined spectrophotometrically with PG T160 VIS Spectrophotometer at wavelength 620 mm and

anthrone reagent using D-glucose as standard (Dey and Harborn, *1993*). Determination of mannoproteins was done gravimetrically (Liu et al., 2011). Protein was determined according to Lowry method (Lowry et al., 1951). Catalase activity was determined by the method described by Efremova (Efremova et al., 2013).

Statistical processing of the results was performed using calculation of standard errors for average and relative values. Correlation between samples and statistical differences was assessed using t-Student criterion and materiality ($p < 0.05$)

RESULTS AND DISCUSSIONS

In order to establish the time interval taken by the cell under the influence of extra high-frequency millimeter waves to activate its physiological processes, the content of total carbohydrate, mannoproteins, protein, catalase activity was determined.

The determination of cell biomass, mannans, carbohydrate content has demonstrated the positive reaction of yeast strain to the action of irradiation. Following the mathematic calculation, it was obtained biomass content of 5.68- 6.05 g/l, carbohydrate content 29.14 to 39.65% of dry biomass, mannoproteins varied from 11.7 to 13.93 % dry cell mass, which is by 21.0%, 50.58% and 30.06%, respectively, more than the control. Maximum biomass and carbohydrate content was accumulated at the duration of irradiation of 20 minutes and mannoproteins accumulation was established at 10 minutes of irradiation (Fig. 1).

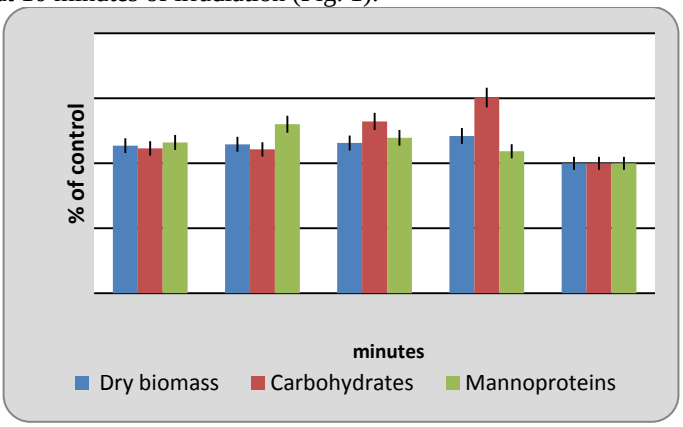

Figure 1. Effect of millimeter wave f = 53.33 GHz on the content of mannoproteins, carbohydrates and biomass *Saccharomyces cerevisiae* CNMN-Y-18 depending on the duration of irradiation

The calculation of linear correlation r (Pearson) for interpreting the biomass and mannoproteins content revealed a strong association. The coefficient of determination $R^2 = 0.926$ or 92.6% is determined by the variation of the other variable values (Figure 2a).

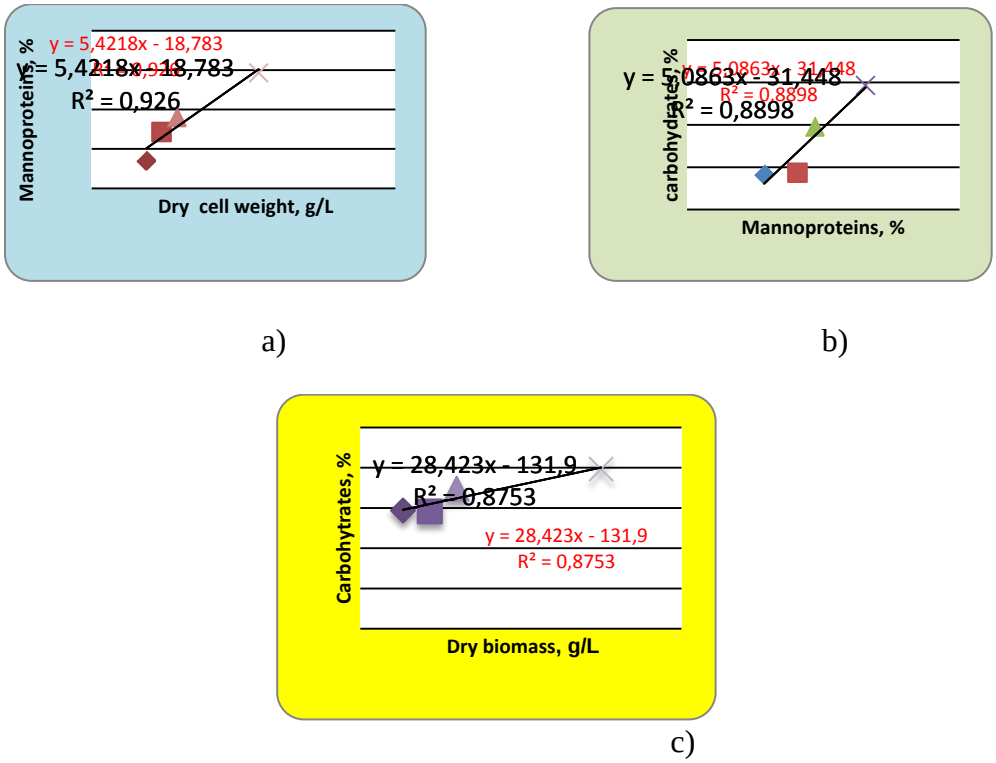

Figure 2. The interdependence between biomass, carbohydrates and mannoproteins content in *Saccharomyces cerevisiae* CNMN-Y-18, irradiated with millimeter wave f = 53.33 GHz.

The analysis of the correlation parameters between mannoproteins and carbohydrates content (fig.2b) established an ascending tendency values that imply variable an ascending tendency of other variables. The bond between them identified as $R^2 = 0.889$ or 88.9%, argues the hypothesis of the existence of real bonds in the base of those can be foretelled values on the base of the regression equation. The possible explanation would be that variables, the carbohydrate and mannoproteins content are influenced by the same phenomenon –millimeter waves treatment.

Calculation of the correlation coefficient for two variables - biomass and carbohydrate content $R^2 = 0.875$ or 87.5% demonstrated strong dependence between biomass and carbohydrate accumulation (Figure 2c).

The data referring to the protein content and catalase activity of *Saccharomyces cerevisiae* CNMN-Y-18 yeast strain are demonstrated in Figure 3. According to the obtained results, the highest values of protein content, as well as the catalase activity in the yeast strain has been determined by the duration of irradiation of 15 min, which was 39.26% and 2936 U/mg of protein, respectively, which was with 33.0% and 34.0% more than non-irradiated sample.

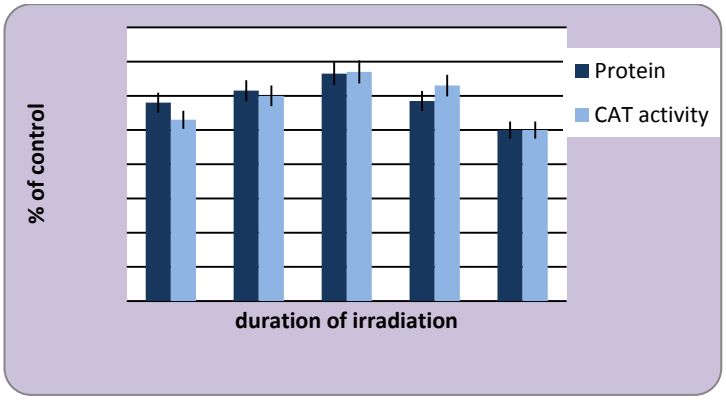

Figure 3. The effect of the duration of action of millimeter wave f = 53.33 GHz on protein content and catalase activity in *Saccharomyces cerevisiae* CNMN-Y-18 yeast strain.

The determination of the correlation between protein content and catalase activity in *Saccharomyces cerevisiae* strain CNMN-Y-18 confirms the existence of dependence between these parameters; correlation coefficient was 58.10% (Figure 4). Therefore, high levels of catalase activity are completely determined by the content of protein. Thus, extremely high frequency radiation has changed microbial metabolism and stimulated production of protein in yeast biomass. Oxidative stress condition induced by the irradiation has been reported to change enzymatic reaction by stimulating of catalase activity.

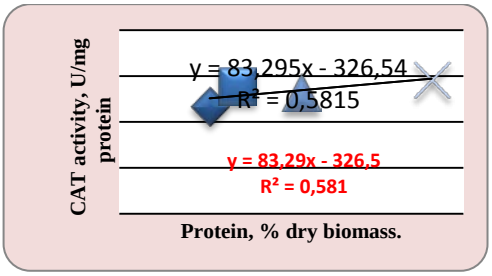

Figure 4. Interdependence between protein content and catalase activity in *Saccharomyces cerevisiae* CNMN-Y-18, irradiated with millimeter waves f = 53.33 GHz.

The analysis of selected indices of *Saccharomyces cerevisiae* CNMN-Y-18 yeast has demonstrated different levels of response to millimeter waves irradiation with different duration of treatment. Summarizing the experimental results it can be revealed that maximum amount of biomass and carbohydrates is accumulated for a irradiation time of 20 minutes. The treatment of yeast cell for 10 minutes provides high content of mannoproteins by 30% compared to the control. It was determined the optimum time for irradiation is 15 minutes whuich determines an increase of protein content and catalase activity in *Saccharomyces cerevisiae* CNMN-Y-18 yeast

strain. Analysis of the interdependence between biomass content, carbohydrates, mannoproteins, protein and catalase activity in irradiated yeast strains has established an ascending tendency of correlation.

According to research results, the new procedure of directed synthesis of mannoproteins at yeasts using extremely high frequency has been proposed. The elaborated procedure of activation of mannans biosynthesis includes the following stages: inoculums obtaining; the irradiation of inoculum with millimeter wave (f = 53.3 GHz) emitted on a continuous basis; subsequently seeding the fermentation medium with obtained sterile inoculum ($2x10^6$ cells ml $^{-1}$) at a concentration of 5%; submerged cultivation under continuous stirring (200 rpm) at 25˚ C for 120 hours. Yeast biomass is then further separated from the culture liquid, then mannoproteins subsequently extracted.

The scheme of the realization of procedure it is presented in figure 5 and includes the following stages:

Stage I. Inoculum obtaining.

The obtaining of 24 hours old inoculums by culturing *Saccharomyces cerevisiae* CNMN-Y-18 yeast in depth in the beer wort on the rotary shaker (200 rpm) at a temperature of 25˚ C.

Stage II. Inoculum irradiation with extremely high frequency.

Irradiation of inoculum with the millimeter wave f = 5,33 GHz for 10 minutes. The obtained sterile inoculum are used for further fermentation.

Stage III. Submerged yeast cultivation.

The cultivation of yeast strain on YPD nutritive medium at a temperature of 25^0C for 96-120 hours. The separation of yeast biomass from the culture liquid by centrifugation 3000 rpm/min for 20 min.

Stage IY. Extraction of mannoproteins.

Spent brewer’ s yeasts (20 g dry weight) were first sieved (mesh diameter 125 mm), then the yeast suspension was centrifugated at 4500 g for 10 min, followed by re-suspension of the deposition in sterile distilled water. The procedure was repeated 5–6 times until the supernatant was clear, then the yeast was purified. After purifying, 1 L of 2% NaOH (w/v) was added to the cell wall sediment. This was placed in a boiling water bath and agitated at 150 rpm/min for 2 h. The preparation was centrifuged and the supernatant was collected. The residue was washed with little deionized water and combined with supernatant extracts. After that, the pH was adjusted to 6.5 with 10% acetic acid, and the supernatant was concentrated to one fifth of the original volume by the evaporation, ethanol was added to supernatant with the aim to precipitate mannoproteins. The precipitated mannoprotein was dissolved in water and centrifuged; the supernatant was precipitated again by the addition of ethanol and recentrifuged. The obtained white sediment was washed twice with ethanol and once with ether, then dried at 70 ˚C

The proposed method provides an increase in mannoproteins content with 26 to 30% compared to the control.

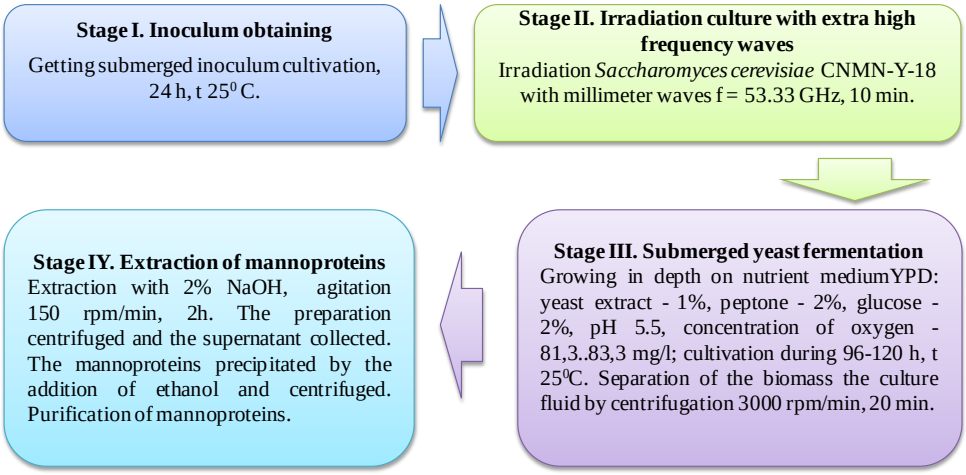

Figure 5. Scheme of performing the process of increasing in mannoproteins content in *Saccharomyces cerevisiae* CNMN-Y-18 by using extremely high frequency.

CONCLUSIONS

Saccharomyces cerevisiae CNMN-Y-18 yeast strain has demonstrated various response to the extremely high frequency waves (f = 53.33 GHz) irradiation. Positive influence on the accumulation of biomass and carbohydrates exhibited irradiation time of 20 minutes, the protein content and catalase activity - after 15 minutes of irradiation and biosynthesis of mannoproteins was activated after 10 minutes of irradiation. High dependence has been established between biomass, carbohydrates, mannoproteins content at selected yeast strain (coefficient of correlation R^2 = 0.875 ... 0.926), which demonstrates that these cellular components are influenced by extremely high frequency. The new procedure of increasing of the biosynthesis of mannoproteins in *Saccharomyces cerevisiae* CNMN-Y-18 yeast strain, based on the use of millimeter waves as the stimulating factor with duration of irradiation for 10 minutes allows its successful employment in the industrial production of mannoproteins.

REFERENCES

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.

Cifra, M., Jelinek, F., Pokorny, J., Janca, R. et al. (2011): *Measurement of Electrical Oscillations of Yeast Cells Membrane at Acoustic Frequencies.* Biological Effects, Medical and Industrial Applications of EM Field. ISMOT Proceedings, Prague, Czech Republic, JUNE 20 -23, 2011, 303-306.

De Vita, A., Croce, R. P., Pinto, I. M. and Bisceglia, B., (2011): *Nonlinear interaction of electromagnetic radiation the cell membrane level: response to stochastic fields.* Progress In Electromagnetics Research B, 33, 45-67.

Dey, P., Harborn, J., (1993): *Methods in Plant Biochemistry*. *Carbohydr*. Academic Press, 2, 529 p.

Efremova, N., Usatîi, A., Molodoi, E. (2013): *Metodă de determinare a activităţii catalazei*. Brevet de invenţie 4205 MD, BOPI nr. 2/2013, 26.

Liu Hong-Zhi, Qiang Wang, Yuan-Yuan Liu, Fang Fang, (2009): *Statistical optimization of culture media and conditions for production of mannan by Saccharomyces cerevisiae.* Biotech. and Bioprocess Engineering, 14(5), 577-583.

Liu Hong-Zhi, Qiang Wang, Yin He, (2011): *Immunoactivities and antineoplastic activities of Saccharomyces cerevisiae mannoprotein.* Carbohydrat polymers, 83, 1690-1695.

Lowry, O., Rosebough, N., Farr, A. et. al., (1951): *Protein measurment with the folin phenol reagent*. J. Biol. Chem., 193, 265-275.

Markkanen Ari. (2009): *Effects of Electromagnetic Fields on Cellular Responses to Agents Causing Oxidative Stress and DNA Damage*. Doctoral dissertation. To be presented by permission of the Faculty of Natural and Environmental Sciences of the University of Kuopio, Medistudia building, University of Kuopio, on Tuesday 16 th June 2009, 53.

Molodoi E., Usatîi A., Efremova N., Fulga L., (2014): *Influența undelor cu frecvență extra înaltă asupra activității biosintetice a levurii Saccharomyces cerevisiae CNMN-Y-18.* Buletinul Academiei de Științe a Moldovei. Științe ale vieții, 2(323), 157-163.

Ruiz-Gomez, M. J, Prieto-Barcia, M. I, Ristori-Bogajo, E., Martinez-Morillo, M., (2004): *Static and 50 Hz magnetic fields of 0.35 and 2.45 mT have no effect on the growth of Saccharomyces cerevisiae*. Bioelectrochemistry, 64, 151– 155.

Usatîi, A., Molodoi, E., Efremova, N., Chiseliţa, N., Borisova, T., Fulga, L., (2013): *Tulpină de drojdii Saccharomyces cerevisiae – producătoare de manani*. Brevet de invenţie 4216 MD, BOPI nr. 4/2013, 24.

Acknowledgements. The research was conducted within the project 11.817.08.19 A, funded by CSSDT of ASM, Moldova.

1 - Institute of Microbiology and Biotechnology of Academy of Sciences of Moldova, Chishinau, Moldova
***** - usatyi.agafia@gmail.com