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THE USING OF MILLIMETER WAVES FOR BIOSYNTHETIC PROCESSES STIMULATION IN *SACCHAROMYCES CEREVISIAE*

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Abstract: The results of influence of three frequencies of electromagnetic radiation of high-frequency range (EMR EHF) on the biosynthesis of carbohydrates, β-glucan, proteins, catalase activity by *Saccharomyces cerevisiae* CNMN -Y-20 yeast strain were analysed. It was established that frequency of f= 53,33 GHz stimulates the biosynthesis of carbohydrates, including β-glucan and frequency of f= 42,19 GHz promotes the increase of protein content and catalase. The indicated frequencies of EMR EHF are offered for the use in the biotechnology of cultivation of yeasts with the purpose to increase biosynthetic properties of yeast strain.

Key words:*Saccharomyces cerevisiae*, millimeter waves, carbohydrates, β-glucan, proteins, catalase activity.

INTRODUCTION

Extra high frequency millimeter waves (ELF, extremely low frequency) are aligned to ionizing radiation type and refers to electromagnetic oscillations ∫ = 30-300 GHz frequency tuning fork holding wavelength λ = 1-10 mm (Markkanen, 2009).

ELF find their wide application in various fields, particularly medicine and biotechnology. An impressive number of scientific research - basic,

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experimental and applied on millimeter electromagnetic field action on microorganisms and laboratory animals, the effect in the treatment of diseases with different etiologies, in various biotechnologies were conducted successfully under implementation in the Russian Federation, Ukraine, Italy, Czech Republic, Australia, Spain, Croatia, Finland, etc. (Cifra et al., 2008, De Vital et al., 2011, Markkanen, 2009, Pirogova et al., 2009, Betskii et al., 2009). Studies show the presence of low frequency effects of millimeter waves (up to 10 mW - cm2) on growth and cell proliferation, enzymes activity, the functioning of cell membranes, and other biological systems.

A milestone in the study of mechanisms of interaction of electromagnetic field in the millimetric range with biological objects, highlighting the structural unity, that reacts directly to external factors (Garkusha et al., 2008, Saveliev et al., 2012). According to the authors biomembranes are the receiving element of millimeter wave at which level occur changes in the oscillation frequency.

In therapeutic practice, usually are used three frequencies: f = 42.25, 53.57, 61.2 GHz (λ=7.1 mm, 53.57 mm, 4.9 mm). If the first two wavelengths were determined experimentally in animal experiments with microorganisms, then the third λ=4.9 mm corresponds to the absorption line of molecular oxygen of air (Betskii et al., 2002). Due to a number of features of millimeter wave interaction with biological objects, their use in biotechnology is very important.

Obviously, to use millimeter waves in biotechnology of direct cultivation of yeasts, to obtain bioactive polysaccharide products and other ones, detailed research is needed to determine their action on the sensitivity of the cells, the regulatory functions of biosynthetic processes, the results have important scientific and practical interest. In light of the presented above we aimed to estimate the influence of millimeter wave with different spectrum of extra high frequency on biosynthetic activity of the strain *Saccharomyces cerevisiae* CNMN-Y-20 and selecting frequencies with positive effects on technological indexes of yeast.

MATERIALS AND METHODS

Research object. As an object of research served *Saccharomyces cerevisiae* strain CNMN-Y-20, producer of β -glucans, preserved in the Laboratory Yeasts Biotechnology and Collection of Nonpathogenic Microorganisms (Chiselita et al., 2010). The strain was obtained from wine sediment, selection method in multiple stages, the wort 7% liquid agarose.

Media and culture conditions. Seed material was obtained by growing yeast strains on wort for 24 hours on shaker (200 rpm.) at a temperature of +25°C. The inoculum was used for millimeter-wave radiation. After exposure to ELF millimeter waves, yeast cells, in an amount of 5%, 2x10^6 cells/ml were inoculated on the liquid medium and grown under the same conditions as the control. Cultivation was carried out in depth capacity 1 liter Erlenmeyer flasks containing 0.2 L YPD broth (1% yeast extract, 2% peptone, 2% glucose, 1 liter water, pH 5) (Aguilar-Uscanga et al. 2003), the duration of cultivation 120 hours at a temperature of +25°C.

Yeast cells were irradiated using the extra high-frequency millimeter КВЧ-НД, RS-232, with the wavelength λ=4.9, 5.6, 7.1 mm, which corresponds to the frequency f = 60.12, 53.33 and 42.19 GHz (maximum 10mW/cm2). The device is certified and permitted for use in medical practice.

Methods. Cell biomass was determined gravimetrically (Liu et al., 2009). As a means for the analysis of carbohydrates in the biomass of yeast were used spectrophotometric techniques using Antron reagent and D-glucose as standard (Dey et Harborne, 1993). The content of β-glucans in the yeast biomass was determined gravimetrically as described (Thammakiti et al., 2004). Protein was determined by the Lowry method (Lowry et al., 1951). Catalase activity was determined by the method (Aebi, 1984, Efremova et al., 2012).

Statistical processing of the results was performed using calculation of standard errors for average and relative values. Were assessed differences between experimental and control using t-Student criterion and materiality "P" (Dospehov, 1985).

RESULTS AND DISCUSSIONS

It is considered that values fluctuations of cell proliferation index directly reflect the intensity of physiological and biochemical reactions, in turn dependent on the speed of fermentation reactions. Reducing or increasing of proliferation index value under the action of various factors, denotes the characteristics of processes, generated by stimulation or braking of cell multiplication. In this context, in order to improve the quality of biosynthesis, are important research related to the yeast biosynthetic peculiarities under the influence of extra high frequency millimeter waves. The commercial interest presents the yeast cell wall polysaccharides, which can be used in food, pharmaceutical, cosmetics.

Evaluation of the action character of extra high frequency millimeter waves was performed on total carbohydrate content, content of β-glucan, proteins, catalase activity, and the production of yeast biomass.
The analysis shows that after 120 hours of cultivation in YPD medium-depth, for some biochemical components investigated were observed accurate differences between the control and exposed to millimeter-wave variations. Stimulating effect of carbohydrate content (16.7% and 43.5%) was found in yeast biomass exposed to frequencies f = 60.12 GHz and f = 53.33 GHz (wavelength 4.9 and 5.6 mm), 20 minutes irradiation time. The results of the analysis of data are presented as histograms that characterize the differences between experimental and control samples indices, expressed as a percentage of control (Figure 1).

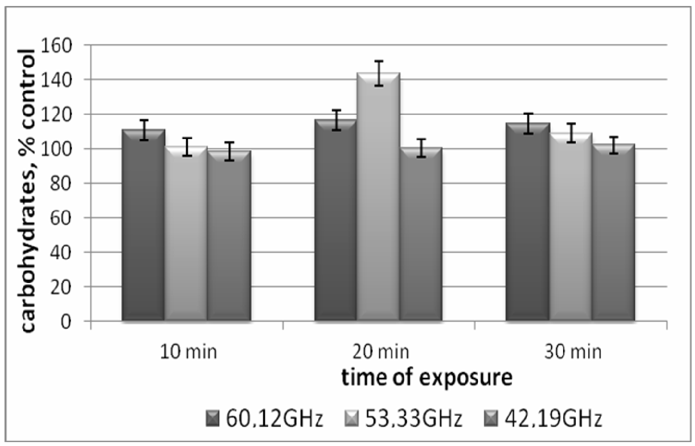

Figure 1. Effect of extra high frequency millimeter waves on carbohydrates content in *Saccharomyces cerevisiae CNMN-Y-20.*

Clause of changes in carbohydrate content determined in yeast biomass would be that these, together with protein molecules in the cell membrane, represent the regulation factor of physical and chemical processes. We assume that the influence of extra high frequency millimeter waves through protein receptors vital functions of the cell changes.
Later was studied another important indicator of the chemical composition of the cell - β-glucan. They have a special significance because of their biomedical immunomodulatory, antiviral, anticancer activities. In combination with other biologically active substances, β-glucan have the ability to reduce the levels of toxins in the digestive tract; the mechanism of

this process is based on the polysaccharide chain complexion toxins with hydrogen bonds.

The survey data showed that the use of millimeter waves in the cultivation of yeast has contributed to the quantitative content of β-glucan. Differences truthful mention the use of millimeter wave frequencies f=60.12 GHz and f=53.33 GHz (λ=4.9 and λ=5.6), the exposure time 10 and 20 minutes. Percentage deviation from control indices are 17.4% and 25.7% for stimulation of β - glucan content (Figure 2). It was found that is missing veritable tend to increasing and to decreasing the glucan content, when the exposure to millimeter waves is 30 minutes, all specific frequencies used. In our view, the biological effect of millimeter waves is temporarily, 20 minutes, more exposure of biological object to radiation does not result in increased biological effect. This phenomenon is confirmed by other researchers, which shows that the biological effect of millimeter waves is an action threshold after appearing plateau effect (Rotaru, 2008, Betskii et al., 2009).

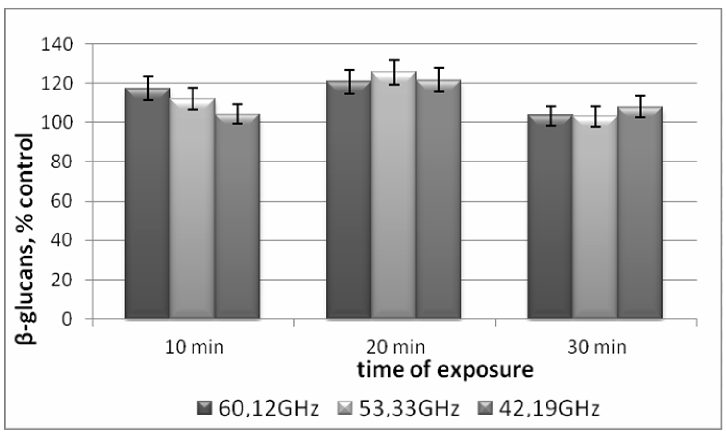

Figure 2. Effect of extra high frequency millimeter waves (*f*=60.12 GHz, 53.33 GHz, 42.19 GHz) on the β-glucans content in *Saccharomyces cerevisiae CNMN-Y-20.*

From the above mentioned it is evident that a significant increase in carbohydrate and β-glucans content in *Saccharomyces cerevisiae* CNMN-Y-20 action occurs at millimeter wave frequencies are 60.12 GHz and 53.33 GHz, applied for 10-20 minutes.

During the complex research millimeter wave influence on the biosynthetic capacity of yeast were carried out investigations regarding protein content and catalase activity. It was observed that exposure of yeast culture during 10 and 20 minutes stimulated tissue regeneration. In yeast variants exhibited frequency f=42.19 GHz, the protein content increased by 41.2 ... 49.9% compared to the control (Figure 3). Increased protein content values were recorded at strain irradiation by millimeter wave frequency 60.12 GHz for 10-20 minutes. The values of protein content increased by 35.6 ... 41.0% compared to the control.

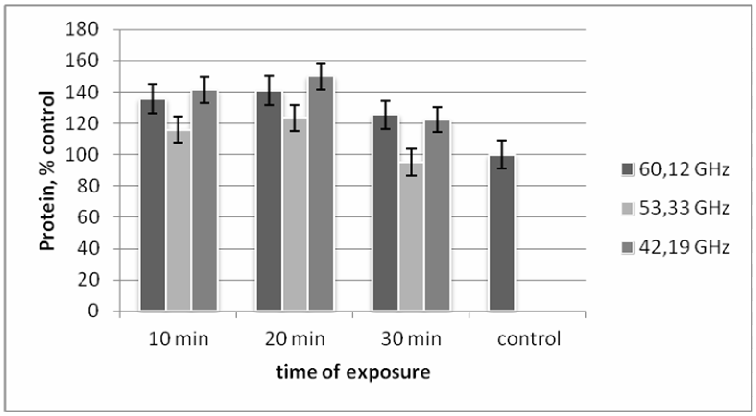

Figure 3. Effect of extra high frequency millimeter waves (*f*=60.12 GHz, 53.33 GHz, 42.19 GHz) on protein content in strain *Saccharomyces cerevisiae* CNMN-Y-20.

Parallel was conducted study of catalase activity, which is one of the most efficient catalysts known, reactions that are essential for life. Catalase reduces the level of hydrogen peroxide to oxidize toxins, phenols or alcohols. The study allowed us to identify the wavelength and duration of the yeast culture exhibition which initiate accelerate formation of catalase. Catalase response is more prompt (34.5 ... 39.4% more than the control) to stress caused by frequency f = 42.19 GHz irradiation lasting up to 30 minutes (Figure 4). The increased amount of catalase recorded in all experimental versions justifies its involvement in the process which produces large amounts of hydrogen peroxide.

In order to increase *Saccharomyces cerevisiae* strain CNMN-Y-20, metabolic activity, knowing the build proteins indices and the catalase activity, we propose to process the growing use of millimeter wave frequency 42.19 GHz (λ=7.1 mm) for 10 to 20 minutes.

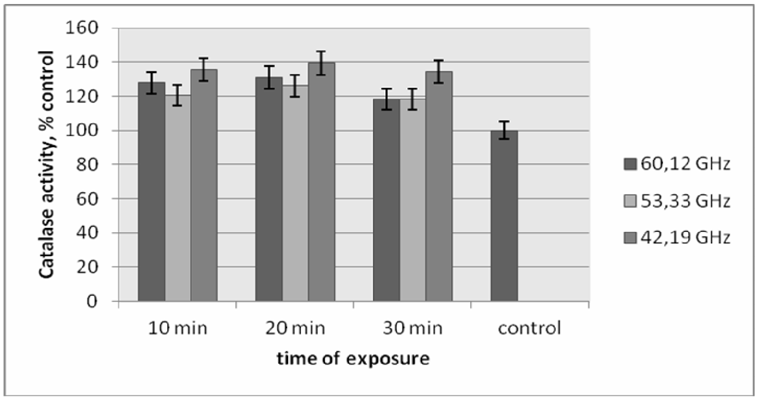

Figure 4. Effect of extra high frequency millimeter waves (f=60.12 GHz, 53.33 GHz, 42.19 GHz) on catalase activity in strain *Saccharomyces cerevisiae* CNMN-Y-20.

Analysing biomass productivity we can mention that examined frequencies issued for 10, 20, 30 minutes does not change essentially quantitative indices. Some results obtained in variants with frequency f=60.12 GHz (wavelength λ=4.9) (10 minutes exposure) or frequency 42.19 (wavelength λ=7.1) (30 minutes exposure) indicates a insignificant decrease in biomass content (Figure 5).

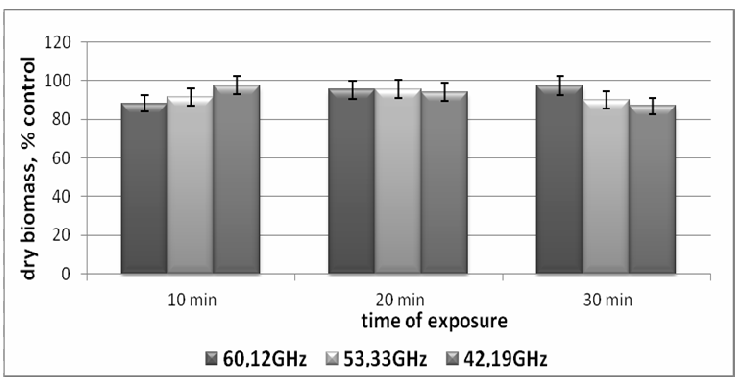

Figure 5. Effect of extra high frequency millimeter (f=60.12 GHz, 53.33 GHz, 42.19 GHz) on biomass accumulation in *Saccharomyces cerevisiae CNMN-Y-20.*

Thus the investigation results indicate dependence of cell functional activity of *Saccharomyces cerevisiae* CNMN-Y-20 strain from extra high-intensity millimeter waves frequency applied in the cultivation of yeast. Frequency f=53.33 GHz has a pronounced effect of stimulating the biosynthesis of total carbohydrates, including polysaccharide β-glucan, and the frequency f=42.19

GHz activates protein formation and catalase. This allows us to mention that extra high-intensity millimeter waves are an important factor of cell metabolism modification; and the examined frequencies can be proposed for yeast cultivation biotechnology to increase biosynthetic performance.

CONCLUSIONS

Experimental results confirm that the extra high-intensity millimeter waves can be proposed as a regulator of the biosynthetic activity of yeast strain *Saccharomyces cerevisiae* CNMN-Y-20. Under the influence of 53.33 GHz millimeter wave frequency and duration of action of 20 minutes strain active accumulates total carbohydrates. The significant increase in the content of β-glucan in the cell wall is produced under the action of 53.33 GHz millimeter wave frequency applied for 10-20 minutes. The biological effect of the millimeter wave is temporarily during 20 minutes, after this time exposure of yeast to radiation does not lead to an increase in biological effect. Optimal regimen to increase protein accumulation and activity of catalase is applying millimeter wave frequency 42.19 GHz (λ=7.1 mm) and irradiation time 10 to 20 minutes.

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