BIOSYNTHETIC POTENTIAL OF *Saccharomyces* YEASTS AT THE TREATMENT WITH EXTREMELY HIGH FREQUENCY MILLIMETER WAVES

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Abstract. Experiment results of the study of extremely high frequency millimeter waves (EHF) of 60.12; 53.33 and 42.19 GHz effect on biomass accumulation and biosynthetic activity of *Saccharomyces cerevisiae* CNMN-Y-20 and *Saccharomyces cerevisiae* CNMN-Y-18 yeast strains – polysaccharides producers deposited in National Collection of Nonpathogenic Microorganims of Institute of Microbiology and Biotechnology of Academy of Sciences of Moldova have been revealed. Duration of treatment of yeast strains in carried out study constituted 10, 20, 30 minutes. Research has demonstrated the efficiency of extremely high frequency electromagnetic radiation for stimulation of biosynthetic processes at yeasts manifested in the increase of bioactive substances content while the stimulation or maintaining at the same level of biomass production.

The irradiation of yeast strains with millimetric waves contributed to the increasing of carbohydrates with 29-43.5%, ß-glucans with 17.4-25.7%, mannans with 11.2-21% and protein content with 26.9-49.9 % as well as to the increasing of catalase activity with 34.5-69.2% compared to the control in dependence on strain, used frequency and duration of irradiation.

Keywords: *Saccharomyces cerevisiae,* biologically active substances, extremely high frequency millimeter waves.

INTRODUCTION

Research regarding the influence of electromagnetic radiation on biological objects is of great scientific interest. According to experimental literature data, mechanism of interaction between millimeter waves and unicellular and multicellular organisms involves vital essential aspects of animals (including humans), plants and microorganisms.

According to some hypotheses concerning these mechanisms, the effect of millimeter waves could be explained by the resonance absorption of electromagnetic radiation at cellular level, as a result of this adaptive cell response was formed. These hypotheses are based on the property of electromagnetic waves to transmit information (external irradiation modulated signals to control vital function of live organisms), thus, some proteic complexes responsible for recovery of cell activity were formed on cellular membranes [10, 38].

Cell membranes, that are responsible for energy storage and biochemical processes regulation by modification of diffusion levels, serve an important role in formation of adaptive reactions as response to external factors [15]. According to literature data, millimeter waves might have beneficial effect on human organism, in this way electromagnetical radiation is succesfully used in the field of medicine for the treatment of a large number of diseases and disorders such as cardiovascular, neurological, urological, ginecological, dermatological, gastrointestinal, dental, ophtalmological, oncological, as well as might protect hematopoetic system eliminating chemotherapy side effects [5-8, 19, 20, 23, 26]. Furthermore, it is well known immunostimulatory, immunomodulatory and anti-inflammatory impact of millimeter waves on human organism [15, 16, 25].

Other fields of application of extremely high frequence millimeter waves are the following – veterinary (treatment of animals diseases), agriculture (plant protection, ameliorating seed germination) [32] and biotechnology [3, 4, 33]. There are various studies regarding the effect of millimeter waves on microorganisms of different taxonomic groups. Thus, recent research have revealed the influence of electromagnetic radiation on photosynthetic organisms-cyanobacteria, micro - and macroalgae [28, 34], bacteria [30], yeasts [14, 17, 21]. Time, frequency, degree of regularity of irradiation of microorganisms by millimeter waves, as well as physiological changes that result from this interaction were established. Also, research has revealed the effect of irradiation on cell proliferation, growth and accumulation of biomass, enzymes activity, function of cell membranes, membrane transport and secretion of bioactive substances at prokaryotic and eukaryotic organisms.

Given the fact that microorganisms present an integral part of biotechnological processes widely used in food industry, agriculture, biotechnology and other, stimulation of biomass accumulation and biosynthesis of bioactive substances with the use of physical factors, enhancing technological capabilities and optimization of cultivation process are of great practical importance.

Regulation of metabolic processes in microbial cultures might be effectuated by utilization of millimeter waves and selection of irradiation parameters. Moreover, the utilization of millimeter waves doesn't have any harmful impact on environment, does not result in other toxic residues, is harmless for biological systems and human life [2, 26, 27].

Whereas extremely high frequency millimeter waves have significant beneficial effect on various biological objects and biosynthetic processes, the aim of this research was to evaluate the effect of this type of irradiation on biosynthetic activity of yeast strains *S. cerevisiae* CNMN-Y-20 and *S. cerevisiae* CNMN-Y-18 in dependence on used frequency and duration of

irradiation by the estimation of biochemical content and catalase activity.

MATERIAL AND METHODS

Research object. As an objects of research served *Saccharomyces cerevisiae* strain CNMN-Y-20, producer of β –glucans [9] and *Saccharomyces cerevisiae* strain CNMN-Y-18, producer of mannans [36], preserved in the Laboratory Yeasts Biotechnology and Collection of Nonpathogenic Microorganisms. The strains were obtained from wine sediment, by the selection method in multiple stages, on the wort 7% liquid agarose.

Media and culture conditions. Seed material was obtained by growing yeast strains on beer wort for 24 hours on shaker (200 rpm.) at a temperature of +25ºC. The inoculum was used for millimeter-wave radiation. After exposure to EHF millimeter waves, yeast cells, in an amount of 5%, $2x10^6$ cells/ml were inoculated in the liquid medium and grown under the same conditions as the control. Cultivation was carried out in 1 liter Erlenmeyer flask containing 0.2 L YPD nutritive medium (1% yeast extract, 2% peptone, 2% glucose, 1 liter water, pH - 5 [1], the duration of cultivation for120 hours at the temperature of +25ºC.

Yeast cells were irradiated using the extra high-frequency millimeter generator KVC-ND, 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 [22]. 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 [11]. The content of β-glucans and mannans in the yeast biomass was determined gravimetrically as described [35]. Protein was determined by the Lowry method [24]. Catalase activity was determined by the method [13].

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 [12].

RESULTS

Because of selected yeast strains *S. cerevisiae* CNMN-Y-20 *and S. cerevisiae* CNMN-Y-18 have biotechnological practical importance and serve as potential producers of β-glucans and mannoproteines, the evaluation of influence of extremely high frequency millimetric waves on biosynthetic activity of *S. cerevisiae* CNMN-Y-20 and *S. cerevisiae* CNMN-Y-18 on cell biomass accumulation, carbohydrates, proteins, β-glucans and mannoproteines content as well as catalase activity.

Analyzing biomass productivity, it can be mentioned that examined frequencies applied for 10, 20, 30 minutes did not change essentially quantitative indices of biomass at *S. cerevisiae* CNMN-Y-20. Some results obtained in variants with frequency 60.12 GHz (wavelength λ=4.9) (10 minutes exposure) and 42.19 GHz (wavelength λ=7.1) (30 minutes exposure) indicated insignificant decrease in biomass content.

The experimental results regarding the influence of millimetric waves on productivity at *S. cerevisiae* CNMN-Y-18 have demonstrated the increase of biomass indices. Maximum values of biomass accumulation (6.02-6.50 g/l dry weight) were obtained at frequency 53.33 GHz, interval of irradiation for 10-20 minutes that was with 18-23% more than control (Fig. 1).

Thus, according to obtained results referring to the effect of millimeter waves on carbohydrate synthesis in yeasts at the cultivation on YPD medium for 120 hours, can be indicated the activization of biosynthetic process at the exposure to radiation with frequency 53.33 GHz for 20-30 minutes. So, in this condition, content of carbohydrates in irradiated was increased with 43.5% at *S. cerevisiae* CNMN-Y-20 and with 29-36% at *S. cerevisiae* CNMN-Y-18, compared to the control (Fig. 2).

The significant modifications of polysaccharide components of cell wall have been revealed. It was established that yeast cells are sensitive to radiation during 10-20 minute of irradiation. Content of β-

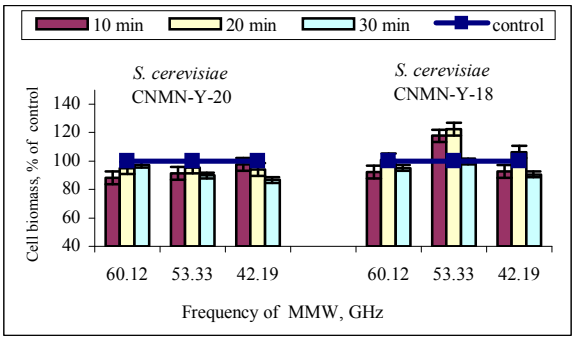

Figure 1. Effect of extremely high frequency millimeter waves (*f*=60.12 GHz; 53.33 GHz, 42.19 GHz) on biomass accumulation in *S. cerevisiae* CNMN-Y-20 and *S. cerevisiae* CNMN-Y-18

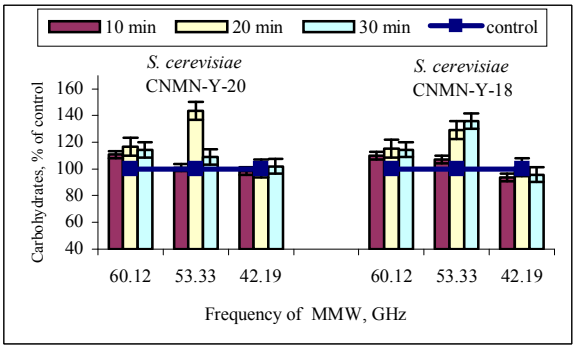

Figure 2. Effect of extremely high frequency millimeter waves on carbohydrates content in *S. cerevisiae* CNMN-Y-20 and *S. cerevisiae* CNMN-Y-18

glucans at *S. cerevisiae* CNMN-Y-20 strain was increased with 17.4 and 25.7% compared to the control, at irradiation with frequency 60.12 GHz (λ=4.9) and 53.33 GHz (λ=5.6), respectively. Moreover, essential alterations of mannoprotein content until 21% of dry biomass in *S. cerevisiae* CNMN-Y-18 at frequency 53.33 GHz (λ=5.6), duration of irradiation 10 minutes (Fig. 3).

Given the fact that proteins possess extended-spectrum functions in live organisms, catalyze various biochemical reactions, assure the cell integrity (as cell wall proteins), ensure immune and autoimmune response of organism, these can serve important indices for the evaluation of the influence of external factors on the cell.

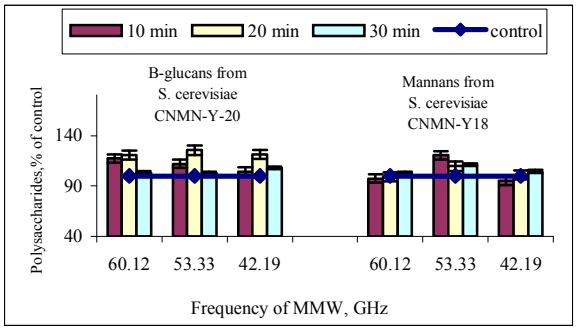

Figure 3. Effect of extremely high frequency millimeter waves (*f*=60.12 GHz; 53.33 GHz, 42.19 GHz) on the β-glucans content in *S. cerevisiae* CNMN-Y-20 and mannans content in *S. cerevisiae* CNMN-Y-18

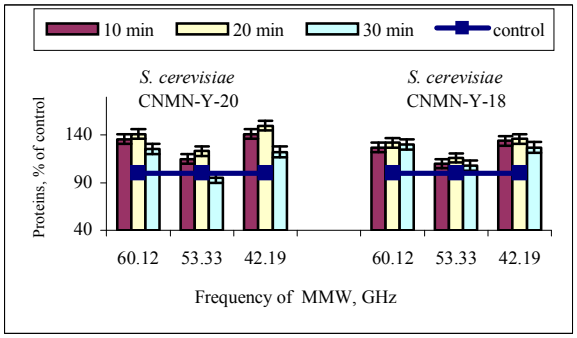

Figure 4. Effect of extremely high frequency millimeter waves (*f*=60.12 GHz; 53.33 GHz, 42.19 GHz) on protein content in strain *S. cerevisiae* CNMN-Y-20 and *S. cerevisiae* CNMN-Y-18

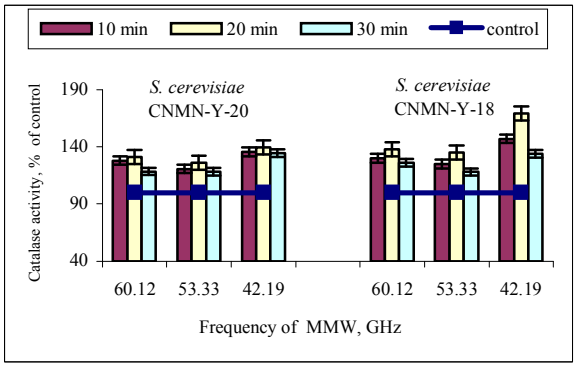

Figure 5. Effect of extremely high frequency millimeter waves (*f*=60.12 GHz; 53.33 GHz; 42.19 GHz) on catalase activity in strain *S. cerevisiae* CNMN-Y-20 and *S. cerevisiae* CNMN-Y-18

The studies carried out on yeast strains exposed to the action of electromagnetic radiation of different frequencies, in dependence on the duration, have demonstrated stimulating effect produced by the irradiation on protein synthesis. This way, the exposure of *S. cerevisiae* CNMN-Y-20 strain to radiation with millimetric waves with the frequency of 60.12 GHz and 42.19 GHz, for 10 - 20 minutes contributed to the increase of protein content with 35.6–41.0% and 41.2–49.9% compared to control, respectively. The response of *S. cerevisiae* CNMN-Y-18 to the action of millimetric waves was identical with other yeast strain, protein content was increased with 26.9-35.6% compared to control by the irradiation with millimetric waves of 60.12 GHz and 42.19 GHz (Fig . 4).

Along with protein content, catalase activity in *S. cerevisiae* CNMN-Y-20 and *S. cerevisiae* CNMN-Y-18 yeast strains has been studied. The significant increase in catalase activity was established for both culture in all variants of irradiation. The maximum increase with 34.5 ... 39.4% compared to control at *S. cerevisiae* CNMN-Y-20 and 42.2 … 69.2% compared to control at *S. cerevisiae* CNMN-Y-18 was obtained in the variants exposed to irradiation with frequency 42.19 GHz, for 10-20 minutes (Fig. 5).

Thus, study of the influence of extremely high frequency millimeter waves with frequency of 60.12 GHz and 42.19 GHz on biosynthetic potential of *S. cerevisiae* CNMN-Y-20 and *S. cerevisiae* CNMN-Y-18 yeast strains allowed to evaluate, for the first time, the action of millimeter waves on the process of cultivation of yeasts and the possibility of their application as stimulating factor for biosynthetic activity of yeast strains with biotechnological importance.

DISCUSSION

Besides the use of the chemical factors, an excellent and actual modality to stimulate the growth of microorganisms and biosynthesis of bioactive substances is the use of physical factors, that might influence the physiological activity of live organisms. At the present time, the possibility of utilization of electromagnatic radiation as stimulator of biosynthetic processes is of great interest. Especially, electromagnetic waves influence on cell membranes. It is well-known, that yeast cell wall is composed mostly of some types of polysaccharides, the basic components are β-glucans and mannoproteins.

In this paper is presented synthesis of study of the effect of extremely high frequency millimetric waves on biomass productivity, biosynthetic activity and enzymatic activity of *Saccharomyces* yeasts strains – polysaccharide producers.

According to experiment results, treatment of yeasts strains with electromagnetic radiation has variable effect on biomass production which depends on the strain, frequency of wave and duration of irradiation. The essential increase of productivity of *S.*

cerevisiae CNMN-Y-18 was established in case irradiation with millimetric waves with frequency 53.33 GHz for 10-20 minutes. Also, an decrease of productivity has been revealed at the irradiation with millimetric waves with other frequencies. These results are similar with the results obtained of other researchers, according to which extremely high frequency millimetric waves might have the stimulating and inhibiting effects on parameters of microbial cultures in dependence on frequency and duration of irradiation. Analysis of scientific papers on identical theme have demonstrated stimulating effect of millimetric waves with frequency 61.8 and 54.17 GHz on biomass production of some *S. cerevisiae* yeast strains [2 ,37].

Scientific investigations with the utilization of NMR relaxometry methods revealed that electromagnetic radiation might influence on biopolymers of the cell wall and cytoplasmic membranes, which are sensible to the frequency of millimetric waves [2], effect was confirmed by present research and was expressed by the stimulating of total carbohydrates biosynthesis at both cultures in the same interval of frequencies 53.33-60.12 GHz and, also, of polysaccharides of cell wall. In this way, the stimulating of ß-glucans synthesis at *S. cerevisiae* CNMN-Y-20 was produced regardless of the used frequency of millimetric waves, but depending on the duration of irradiation. It was established that biosynthesis of mannans in *S. cerevisiae* CNMN-Y-18 depends on used frequency (53.33 GHz).

Furthermore, significant increase in catalase activity at some yeast strains treated with millimetric waves could be explained by fact that the exposure to electromagnetic radiation might cause various alterations induced by free radicals [31], catalase being the antioxidant defense against oxidative stress. All used frequencies of millimeter waves has had an stimulating effect on catalase activity at *S. cerevisiae* CNMN-Y-20 and *S. cerevisiae* CNMN-Y-18 yeast strains, although maximal increase of this enzyme activity was obtained at the irradiation of yeast cells with frequency 42.19 GHz. Similar results were obtained by other researchers, according to those, activity of some enzymes at *Saccharomyces* yeasts was increased in interval of frequencies 53.77-54.57 GHz with maximal values at 54.17 GHz [14] and modification of enzymatic activity in interval of frequencies of 47-67 GHz [18]. According to literature data, EMF exposure caused significant increase in activity of enzymatic antioxidant. Thus, catalase activity was increased in animal cells after treatment with electromagnetic fields [39]. According to other literature data, after soybean seeds were treated by various magnetic fields and time periods, the activities of superoxide dismutase and catalase were significantly increased [29].

The significant increase of protein content was established at the treatment of both culture with millimetric waves with frequency 60.12 and 42.19 GHz.

Thus, it can be noted that irradiation of yeast cells with electromagnetic radiation induced alteration in metabolism, has had stimulating or inhibiting effects on biosynthetic potential, depending on the duration of irradiation.

The experiment results of this research would essentially contribute to the elaboration of technologies for cultivation of *S. cerevisiae* CNMN-Y-20 and *S. cerevisiae* CNMN-Y-18 yeast strains and for obtaining of polysaccharides.

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