EXPERIMENTAL STUDY ON EXTREMELY HIGH FREQUENCY WAVES REGULATION OF PROTEIN CONTENT AND CATALASE ACTIVITY IN *Saccharomyces* STRAINS

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Abstract. This paper presents the results of a study concerning the determination of the effect of extremely high frequency wave's treatment on protein content and the activity of one of the most efficient antioxidant enzyme catalase in selected wine yeast strains. It was established that the highest values of protein, as likewise of catalase activity in *Saccharomyces cerevisiae* CNMN-Y-18 and *Saccharomyces cerevisiae* CNMN-Y-20 were determined at the optimal time of irradiation – 15 min. Thus, indices of protein varied from 39.26 to 41.00% dry biomass. Catalase activity was increased with 34.00 and 38.00%, respectively, more than control. It was found that double irradiation has had positive effect on protein content in two selected yeast strains. Thus, it can be proposed that enzyme activity and protein accumulation can be modulated by the treatment of millimeter waves with specific frequencies.

Keywords: *Saccharomyces cerevisiae;* antioxidant enzyme; catalase; extremely high frequency waves, irradiation.

INTRODUCTION

Along with traditional applications for radiolocation and mobile communication facilities, there are some special field of millimeter wave's application in biology and medicine [18, 20]. The determination of the effect of extremely high frequency (EHF) waves on biosynthetic activity of microorganisms is one of the areas of interest for modern biotechnology. Extremely high frequency waves are electromagnetic waves with the frequency of 30 - 300 GHz that cause different biological effects [23].

Interaction between various type of electromagnetic radiation (EMR) and microorganisms has attracted the interest of scientists [26]. At present, EHF radiation is used in different microbiological applications. EHF attracted great interest among researchers due to the absence of harmful side effects.

Millimeter waves were reported to have a beneficial effect on the microbial activity. The stimulating effect of EHF on growth of actinomycetes *Streptomyces* and cyanobacterium *Anabaena variabilis* has been established [16]. Effect of millimeter waves on cell grown rate and modification of biochemical composition was established in *Bacillus subtilis* [14], *Saccharomyces cereviseae* [3, 10-11, 30], *Candida albicans* [29], *Spirulina platensis* [25].

Various studies demonstrated that electromagnetic fields affect some biochemical processes and result in changing some serum biochemical parameters and enzymes. According to the literature data, magnetic fields enhanced the activity of proteins and enzymes. A brief, low-intensity millimeter wave's exposure can change cell growth and proliferation rates, activity of enzymes, it can alleviate stress reactions [20]. One of the study revealed that lactate dehydrogenase enzymatic activity can be increased by the using of low power microwaves [21, 31]. It was demonstrated that electromagnetic radiation can alter catalase activity [21].

Yeasts are the most promising research objects in the field of biotechnology and microbiology. *Saccharomyces cereviseae* yeasts serve as a widely used biotechnological object as well as a eukaryotic model system [9]. The production of *Saccharomyces* yeast biomass rich in valuable biologically active substances is advantageous due to nontoxic nature and high productivity. Yeasts have rather high growth rates and processes of their metabolism are already well studied enough. Other important aspect is the utilization of culture mediums based on low cost substrates with high yield.

In this paper *Saccharomyces cerevisiae* yeast was chosen for investigation. It is known that these yeasts are frequently used as complementary protein source. *Saccharomyces* also can be considered as a source of antioxidant enzymes, such as catalase (CAT) [6, 8, 13, 17]. It serves as primary line of defense in destroying free radicals. Catalase is one of the most efficient antioxidant enzymes known [28].

It is important to study the reaction of *Saccharomyces* yeasts to electromagnetic radiation. It is known that live organisms activate antioxidant systems as response to the influence of stress condition [4, 19, 27]. Some authors suggested that microwave radiation nonthermally induced different biological effects by changing the protein structures by differentially partitioning the ions and altering the rates and directions of biochemical reactions [24]. One of possible mechanisms of irradiation action on life organisms is an induction with the help of transitions between different isomeric states of biomolecules [23].

Thus, can be proposed that enzyme activity and protein accumulation can be modulated by the treatment with millimeter waves with specific frequencies. **Aim of study**: to investigate time exposure and mode of irradiation dependence of EHF radiation on protein content and catalase activity of two wine yeast strains.

MATERIALS AND METHODS

Strains. For the investigation were used two wine yeast strains - *Saccharomyces cerevisiae* CNMN-Y-18 and *Saccharomyces cerevisiae* CNMN-Y-20 from National Collection of Nonpathogenic Microorganims of Institute of Microbiology and Biotechnology of Academy of Sciences of Moldova.

Medium. Cultivation of yeasts was effectuated on the nutritive medium YPD with the following composition (g/L): glucose - 20.0, peptone - 20.0, yeast extract - 10.0 [2]. YPD medium is widely used for *Saccharomyces* yeast studies and it is optimal for investigation of antioxidant enzymes.

Culture conditions. Seed material was obtained by growing yeast strains on beer wort for 24 hours. The inoculum was used for millimeter-wave radiation. Yeast cells were irradiated using the extra high-frequency millimeter generator КВЧ-НД, RS-232, with the frequency f = 53.33 GHz. The device was certified for use in medical and biological practice.

After exposure to EHF treatment yeast cells were introduced in the liquid medium YPD for further cultivation. Cultivation was carried out in Erlenmayer flasks capacity 1L, containing 0.2 L of nutritive medium at 180-200 rpm agitation rate, at temperature of 23…25ºC for 120 hours.

Protein content was determined spectrophotometrically by Lowry et al. [15].

The determination of catalase activity where the disappearance of hydrogen peroxide is followed spectrophotometrically at a wavelength of 240 nm was effectuated according to Aebi [1] in modification of [7].

RESULTS

The choice of frequency range is conditioned by scientific data and previously effectuated studies. So, the frequency of 53.3 GHz was selected for further investigations.

First, in the initial stage, the influence of EHF waves with frequency of 53.3 GHz on protein content and catalase activity in biomass of two wine yeast strains *Saccharomyces cerevisiae* CNMN-Y-18 and *Saccharomyces cerevisiae* CNMN-Y-20 in dependence on time of irradiation has been studied. Results are presented below (Fig. 1).

It was established that the highest values of protein, as likewise of catalase activity in *Saccharomyces cerevisiae* CNMN-Y-18 and *Saccharomyces cerevisiae* CNMN-Y-20 were determined at the optimal time of irradiation – 15 min. Thus, indices of protein varied from 39.26 to 41.00% dry biomass what is with 33.00 and 34.00% more than control unirradiated sample. Catalase activity was increased with 34.00 and 38.00% more than control. It is necessary to mention that the increase of the duration of irradiation up to 20 min contributed to the decrease of the content of protein and catalase activity, nevertheless protein values were more than of control.

Next, the study of the influence of EHF waves with selected frequency on protein content and catalase activity in wine yeast strains was carried out in dependence on mode of irradiation (single and double). Single and double irradiation of yeast inoculum was effectuated during the 10 and 20 minutes. The experimental results regarding the following study are presented in Fig. 2.

Analyzing obtained results it was found that double irradiation has had positive effect on protein content in two selected yeast strains. Thus, it was demonstrated, that double irradiation during 10 minutes contributed to the superior increase of protein content in *Saccharomyces cerevisiae* CNMN-Y-18 with 40.00% compared to the unirradiated sample. While the utilization of single mode of irradiation at the same 10 minutes allowed to increase protein accumulation process with 28.00% compared to the reference sample. At other yeast strain *Saccharomyces cerevisiae* CNMN-Y-20 the similar effect was observed. Protein content was increased with 20.00% in the case of double irradiation during 10 minutes.

However, the difference between the samples obtained during 10 and 20 min of double treatment was evidentiated. It is necessary to mention that the increasing of time of double irradiation up to 20 minutes led to decrease of protein synthesis in both strains. Although, protein values remain at the high level.

The effectuated study has revealed the dependence of CAT activity in selected wine yeast strains on the mode of irradiation. Thus, double mode of irradiation during 10 minutes contributed to the increase in catalase activity with 38.00% and 35.00% more than control in *Saccharomyces cerevisiae* CNMN-Y-18 and *Saccharomyces cerevisiae* CNMN-Y-20, respectively. While in case of single irradiation during 10 minutes catalase activity was increased with 18.00 and 14.00%, respectively.

The dates of the literature confirm that EMR caused the intense formation of free radicals of oxygen that contributes to the activation of enzymatic antioxidant systems [32]. Thus, possible mechanism, involved in catalase activation due to the action of double irradiation at the optimum duration of 10 min on yeast cells includes the formation of new molecules of the enzyme that contributes to the reduction of increasing number of hydrogen peroxide because of oxidative stress.

According to the effectuated experiments, the increase of duration of double irradiation up to 20 minutes led to the inhibition of catalase activity at both yeast strains. This effect can be explained by fact that double irradiation at 20 min has induced strong oxidativ stress that completely inhibited CAT activity in this case.

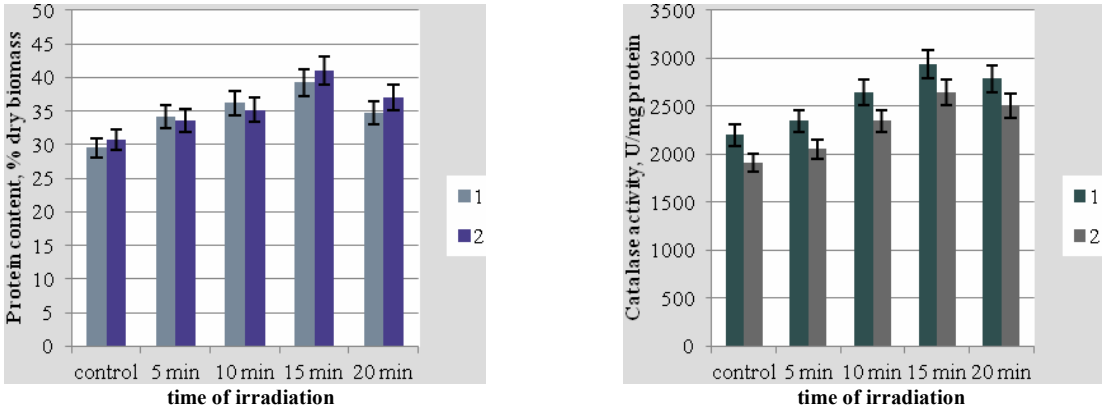

Figure 1. The determination of protein content and catalase activity at 1 - *S. cerevisiae* CNMN-Y-18 and 2 - *S. cerevisiae* CNMN-Y-20 in dependence on time of irradiation

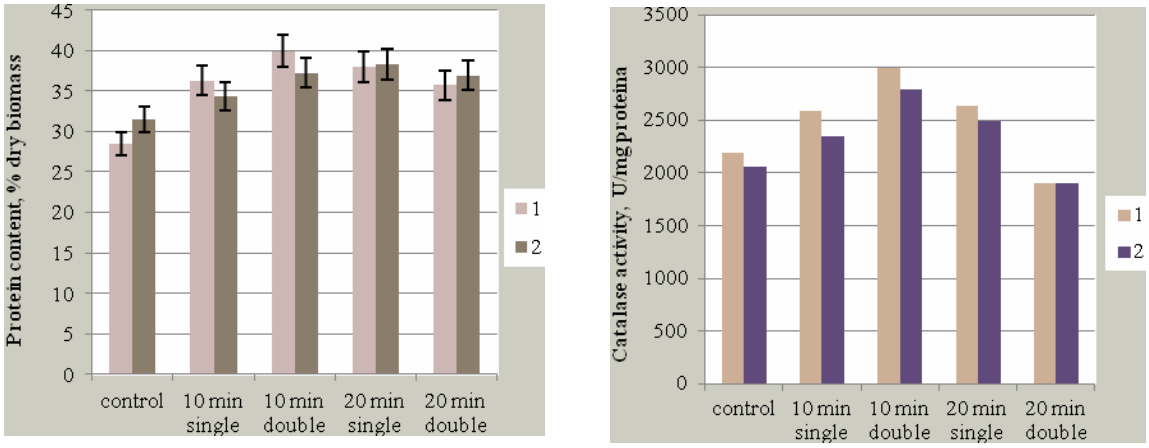

Figure 2. The effect of EHF on protein content and catalase activity in dependence on mode of irradiation at 1 - *S. cerevisiae* CNMN-Y-18 and 2 - *S. cerevisiae* CNMN-Y-20

These results are similar with the dates of scientific literature, according to which, the activity of antioxidant enzymes and protein content depend on oxidativ stress [12].

Thus, it was demonstrated that extremely high frequency waves can be used as stimulators of protein accumulation process in *Saccharomyces* yeast, as well as regulators of catalase enzyme activity.

DISCUSSION

In this study, protein content and one of the efficient enzymes of the defense system - catalase activity was investigated under EHF radiation. Stress condition has been reported to change enzymatic reaction by stimulating of catalase activity.

Analyzing obtained results of studies of the action of electromagnetic radiation on the biological subjects of different level of organization, it can be noted that most if not all, effects can be explained by a change in the functional activity of proteins. Studies have indicated that MMW radiation may alter the structural and functional properties of membranes [21]. Physical actions of EM fields may regulate the rate and the amount of product of biochemical reactions.

It is known that excess of ROS (reactive oxygen species) are scavenged by protective systems. The obtained results coincide with others data, according to which the effect of the electromagnetic radiation of the millimeter range of extremely high frequencies conducts to an increase in the activity of catalase in strains *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* most explicit with 45-minutes exposure [22]. According to literature data, EMF exposure caused significant increase in activity of enzymatic antioxidant. Thus, CAT activity was increased in animal cells (preadipocyte cell culture) after treatment with electromagnetic fields [32]. After soybean seeds were treated by various magnetic fields and time periods, the activities of superoxide dismutase and catalase were significantly increased [22].

According to obtained data, the hypothesis that EHF increases free radicals generation has been proposed. Exposure to electromagnetic field can lead to the increase in free oxygen radicals. In this investigation it was demonstrated that moderate doses of MMW radiation can significantly enhance oxidative stress tolerance.

In this paper the application of magnetic fields has induced stress condition, inclusive oxidative stress and production of reactive oxygen species. Thus, EHF radiation has changed microbial

metabolism and stimulated production of protein in yeast biomass. Probably, EHF waves with frequency of 53.3 GHz influenced microbial metabolism and the number of reactive oxygen species, including direct influences on enzyme action.

It is known that catalase enzyme is an important component of antioxidant protection. In present paper it was demonstrated that MM-wave therapy enhances the activity of this enzyme, which increases the degree of cell protection as adaptive response to oxidative stress. The increase of antioxidant enzyme levels could be a key mechanism in reducing of the cellular damage.

Relevance of investigations is determined by the wide range of application of catalase due to the high antioxidant activity. Protein extracts on the base of antioxidant enzymes from *Saccharomyces* strains could be used for the obtaining of antioxidant preparations for further utilization in pharmaceutical industry and cosmetology.

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