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# MICROORGANISMS AS A MODEL SYSTEM FOR STUDYING THE BIOLOGICAL EFFECTS OF ELECTROMAGNETIC NON-IONIZING RADIATION

**Abstract:** *The safe use of generators with non-ionizing radiation is becoming more relevant due to their intensive propagation in everyday life and the results of researches, which demonstrate their possible negative health effect. Microorganisms are convenient objects for studying the effect of various stress factors, including EMF. In the yeast Saccharomyces cerevisiae (the model organism for the studies of eukaryotic cells behavior),we showed changes of physiological, biochemical, cytological, morphological and genetic parameters under the influence of electromagnetic radiation of EHF, UHF, VHF and generated with video display terminals. Similar effect was also observed in animal tissue cultures (HEp, pig testicular cells, mouse fibroblasts) during these experiments. The results obtained in our research were the basis for the development of the biosensor systems to visualize biological effects of non-ionizing electromagnetic radiation (Patent UA). An increase of luminescence value was detected in photobacteria after treatment with EHF and VHF. These results showed that genetic structures containing the lux-gene can serve as biomarkers to report/prove biological action of non-ionizing radiation. Studies using microorganisms are useful not only for the detection of biological effects and mechanisms of action of non-ionizing electromagnetic radiation, but also have a great practical importance in environmental monitoring, and standardization of EMFs exposure;they are also useful for testing devices designed to provide protection against the possible negative effects of EMFs.*

**Key words:** non-ionizing radiation, model organism, *Saccharomyces cerevisiae*, biosensor.

## INTRODUCTION

Radiofrequency and microwave electromagnetic fields of anthropogenic origin are one of the most extensively propagating factors affecting environment. Their effects on biological processes and, in particular, on human health are of great interest and are discussed intensively. Several theories were proposed attempting to explain mechanisms of biological effects of this factor [1, 2]; however, despite of a huge amount of researches and several special WHO research projects, the question is still open. More and more researchers point out that using of multi-cellular organisms as a laboratory model in the experiments leads to problems. The complexity of these organisms is a problem for objective evaluation of the biological effects of the EMFs and understanding of the mechanisms of interaction between living matter and physical force. In our days, "laboratory studies on cells aim to elucidate the fundamental underlying mechanisms that link electromagnetic field exposure to biological effects. They try to identify mechanisms based on molecular or cellular changes that are brought about by the electromagnetic field - such a change would provide clues to how a physical force is converted into a biological action ..." (cited from WHO webpage "On Health Effects of EMFs", 2011). Among different model systems, the baker's yeast *Saccharomyces cerevisiae* is of great importance. This yeast is the most well studied and widely used organism to study life processes of eukaryotic organisms at different levels, from molecular and genetic to physiological and even social life [3]. A lot of similarities with other eukaryotic multi-cellular organisms and especially with the human cells suggested this yeast as a widely accepted model system for studying eukaryotic organisms [4].

In our researches we used *Saccharomyces cerevisiae* to study effects of EMFs (VHF (40.68MHz), UHF (1800MHz) and EHF (37GHz)) and radiation emitted by video display terminals (computer monitors with cathode-ray tube and TFT). In the current manuscript we only compare effects of the VHF and UHF EMFs. We discuss the impact of EMFs on the physiological, biochemical and genomic aspects of life processes of cells depending on the type of EMF. The effects were compared to other yeasts like *Shizosaccharomyces pombe* (a model organism for molecular and genetic

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studies) and several *Candida* species, among which are pathogenic and semi-pathogenic ones that cause candidiasis in humans. The role of cell cycle phase, membrane integrity, dehydrogenase activity, polyphosphate metabolism and genome instability were evaluated as potential stairs of intracellular transformation of EMFs signals.

Comparative studies about the effects of RF-EMFs on the *S. cerevisiae* yeast and multi-cellular organisms (rats) led to the use of *S. cerevisiae* cells as an adequate sensing element in the biosensor devices.

## PHYSIOLOGICAL AND BIOCHEMICAL IMPACT OF RF-EMFs

The main problem of physiological studies of whole multi-cellular organisms (such as rats, mouse, drosophila, etc.) or their cell/tissue is the complexity of the systems. There are a lot of unknown variables difficult to be established, and some problems with their cultivation. In contrast to these models, yeasts can be easily standardized and provide variations of different factors depending on the experimental needs.

### Yeast population growth and cell cycle effects of RF-EMFs

We showed that effects of the VHF (40.68MHz, 15W and 30W) and UHF (1800MHz with EFD of 10-50 mkW/cm$^2$, 100-200 mkW/cm$^2$ and 1000 mkW/cm$^2$) EMFs depend on the treatment peculiarities and especially on the duration of exposure. Short term exposure (from 5 min to 5 hours) of the non-synchronized yeast population resulted in stochastic effects onto the steichiometric and kinetic parameters of the yeast growth [5]. The cell cycle examination after the short term EMF exposure revealed an increased number of cells in mitosis. This effect disappeared after 4 hours of cultivation without EMF treatment. These results indicate that the effects of EMFs on the yeast may specifically depend on the cell cycle phase.

During long-term irradiation, each cell was exposed to an EMF through the life span (in all cell cycle phases) and as a result the effects of EMF were visible. The treatment of yeasts with EMFs of 1800MHz during 2 days and 6 weeks resulted in several changes in the parameters of their growth: both the specific growth rate and the metabolic coefficient increased. On the contrary, the economic coefficient was unaffected. This parameter indicates that the metabolic processes responsible for the growth and proliferation were enhanced. The long-term EMF effects did not return to the control rates even after 300 generations without irradiation.

The level of general activity of dehydrogenases rose in irradiated cells in accordance with the EMFs (VHF and UHF) exposure and showed good relation to the growth parameters. In the same way activities of some specific dehydrogenases (DHO) of the glycolysis and citric acid cycle (glucose-6-phosphate DHO, alpha-ketoglutarate DHO, succinate DHO) increased under the influence of the EMFs. This indicated the activation of metabolic processes in the irradiated cells. Though it should be noted that such effect of EMFs depend on the type of yeast, the same tests with other yeasts *S. pombe* and *Candida utilis* showed similar result for the first one and showed decrease of enzymes activities for the second yeast.

### Membrane integrity under action of RF-EMFs

Membrane and cell wall (in case of yeast) are two main barriers between the environment and intracellular life processes. Both of these structures may change in response to the action of environmental factors. A lot of researches indicated the role of specific components of the cell membranes, like $Ca^+$- and other ion-channels, in the cell interaction with varied EMFs. We marked that a treatment with VHF EMFs caused the decrease of membrane permeability, while the zeta-potential left unchanged. The same decrease in membrane permeability was detected for the yeast *S. pombe* and *C. utilis.* The biggest decrase was found in *C. utilis* .

The fatty acid profiles of cellular membranes left unchanged after treatment with VHF EMF, while the sterol composition (and especially ergosterol content) changed significantly. The synthesis of ergosterol did not stop in irradiated cells even under hypotonic conditions (Tab. 1), where it is blocked under normal conditions. The amount increased by 30%. Ergosterol is the main structural element of yeast membranes that determine the membrane stiffness. Therefore, the detected decrease of the yeast membranes permeability could be connected with the increased sterol production and the rise in ergosterol quantity within membranes.

**Table 1.** *Effect of EMFs of 40.68 MHz (30W, 60min) on the yeast S. cerevisiae Y-517 sterol (mkg/ml) composition under hypotonic conditions*

| Sterol | Initial concentration | After 60 min | |
|---|---|---|---|
| | | Control | UHF |
| Lanosterol | 18.6 | 47.6 | 19.4 |
| 4,4-dimethyl-zymosterol | 1 | 12.8 | 1 |
| Zymosterol | 11.6 | 1 | 13.2 |
| Fecosterol | 1 | 1 | 1 |
| Episterol | 14.7 | 8.3 | 18.8 |
| ergosta-5,7-dien-3β-ol | 1 | 9.7 | 1 |
| **Ergosterol** | **35.4** | **8.1** | **46.4** |
| Total amount | 83.3 | 88.5 | 100.8 |

### Phosphate metabolism impact on the RF-EMFs biological effects

The use of yeast strain *S. cerevisiae* CNX with two inactivated polyphosphatases PPN1 and PPX1 helped to evaluate the role of these enzymes in the yeast sensitivity to VHF and UHF EMFs. The mutant strain showed different changes in comparison to it parental strain *S. cerevisiae* CRY and the wild type diploid strain *S. cerevisiae* Y-517. Changes of the growth parameters were more drastic and opposite to those marked for the strains CRY and Y-517, while cell-to-cell interactions were affected slightly, and genome effects were lower. The results indicate that the phosphate metabolism is important for the cellular response to the EMFs action.

## GENOME EFFECTS OF RF-EMFs

In our study, we evaluated the changes in *S. cerevisiae* genome that occurred under the influence of VHF and UHF irradiation using ISSR-markers. Such types of DNA-markers are widely used for molecular-genetic typing of yeast species [6]. It allows the detection of the variability of nucleotide repeats distribution among various strains and species.

Results of PCR with primer to tetranucleotide repeat showed that the size of amplification products varied from 350 bp to 1700 bp with common for all samples 700bp-amplicon. There were no differences observed between PCR-fragments sets of control untreated *S. cerevisiae* strains (Y-517 and CRY), although the level of polymorphic bands among irradiated strains was up to 80%. Patterns of PCR-products obtained by amplification with primer $(GACA)_4$ showed differences between irradiated and non-irradiated *S. cerevisiae* strains. All irradiated strains possessed 1000bp- and 1700bp-amplicons distinguishing them from non-irradiated strains.

It was supposed that irradiation affected the genomic stability of *S. cerevisiae* and caused a re-organization of the genetic material, which was reflected in the differences in amplicon patterns. It might be the consequence of recombination events during repair process.

## COMPARATIVE STUDY OF YEAST AND HIGHER EUKARIOTS (RATS) EFFECTS OF RF-EMFs

Comparative study of the action of the VHF EMF 1800MHz during six weeks on the yeast cells and rats showed a lot of similar effects on both organisms (see Tab. 2). Though, considering methodological peculiarities, the changes in the yeast cells were detectable several weeks earlier than in rats. And what was of special interest, yeast as a model let quite easily to prolong experiment to check reversion of the effects observed just after irradiation.

**Table 2.** *Comparison of the effects of UHF EMF (1800MHz) on the yeast S. cerevisiae and rats*

| Characteristics | Yeast | Rat |
|---|---|---|
| Cytomorphology changes | yes | yes |
| Metabolic activity changes | yes | yes |
| Physiological changes | yes | yes |
| Behavioral changes | n.d. | yes |
| Genome changes | yes | Possibly yes |
| Effects reversion | Not entire | n.d. |

## BIOSENSORS OF NON-IONIZING EMFs

High sensitivity of the yeast *S. cerevisiae* to the different types of EMFs was discovered to be excellent as a sensor element for the development of biosensor device (Patent UA №62414 from 25.08.2011) for the visualization of biological action of non-ionizing EMFs. It was found that the luminescence value of *Photobacterium phosphoreum* is directly proportional to power and duration of exposure with EHF. Electromagnetic radiation (frequency 61.22GHz, power 1mkW, duration 30 min) increase bioluminescence intensity by 36%. Increasing of power up to 10mkW, under the same conditions, led to rise of the luminescence intensity by 43%.

These results show that genetic structures containing the *lux*-gene can serve as biomarkers to validate biological action of non-ionizing radiation.

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## BIOGRAPHY

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# MIKROORGANIZMI KAO MODEL ZA PROUČAVANJE BIOLOŠKIH EFEKATA ELEKTROMAGNETNOG NEJONIZUJUĆEG ZRAČENJA

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**Rezime**: *Pitanje bezbedne upotrebe izvora nejonizujućeg zračenja postaje sve značajnije zbog široke rasprostranjenosti i usled rezultata israživanja koje ukazuju na njihovo štetno dejstvo na zdravlje čoveka. Mikroorganizmi predstavljaju pogodne objekte za proučavanje efekata različitih štetnosti, uključujući dejstvo elektromgnetnog polja. Na primeru Saccharomyces cerevisiae iz kvasca (organizam za proučavanje eukariotskih ćelija), prikazana je promena fizioloških, biohemijskih, citoloških, morfoloških i genetskih parametara pod dejstvom elektromagnetskog zračenja u opsegu EHF, UHF, VHF i elektromagnenih zračenja od video terminala. Slične promene su primećene i kod drugih posmatranih kultura životinjskog tkiva (HEp, ćelije ćelije testisa vepra, fibroblasti miševa) pri sličnim eksperimentima.*

*Rezultati dobijeni ovim istraivanjima predstavljali su osnov za razvoj biosenzorskih sistema za vizuelizaciju biološki efekata nejonizujućeg elektromagnetnog zračenja (Patent UA). Uočen je porast intenziteta luminiscencije fotobakterija posle dejstva EHF i VHF eletromagnetnih zračenja. Ovi rezultati pokazuju da genetske strukture koje sadrže lux-gene mogu da se upotrebe kao biomarkeri za potvrđivanje biološkog dejstva nejonizujućeg zračenja. Istraživanja u kojima se koriste mikroorganizmi su veoma perspektivna ne samo za detekciju bioloških efekata i mehanizama dejstva nejonizujućeg elektromagnetnog zračenja, već imaju i veliki praktični značaj u monitoringu životne sredine, normaizaciji i standardizaciji izloženosti elektromagnetnim poljim, kao i za testiranje uređaja koji se koriste za zaštitu od negativnih dejstva elektromagnetnih polja.*

**Ključne reči:** nejonizujuće zračenje, Saccharomyces cerevisiae, biosenzor.