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Protocols

Epitaxy of the bound water phase on hydrophilic surfaces of biopolymers as key mechanism of microwave radiation effects on living objects

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ABSTRACT

The research investigates the mechanism of microwave radiation effects on biological characteristics and structural-dynamic parameters of a sensor bioluminescence system. The research objects are a sterile growth medium (fish meal hydrolisate) and a bacterial culture. It has been established that irradiation causes changes of the growth medium spectral properties within the range of 200–350 nm. Changes take place in the intensity and character of luminescence, as well as in relaxation parameters of nuclear magnetic resonance, growth characteristics of the bacterial culture, its cellular morphology and surface topology. The research results enabled us to establish the mechanisms of primary molecular processes that occur when the bacterial culture is exposed to microwave radiation. Transformation of the dynamic-structural state of adsorbed water phases on biopolymer surfaces has been found to be the key factor in the mechanism of microwave effects on living and water-containing objects.

1. Introduction

Over the last 20 years there has been significant progress in the methods of changing structures of substances and properties of living systems [1]. Within this process the study of non-ionizing radiation effects on living systems has been developing gradually, involving advanced research methods. Considerable amount of experimental material has been gathered, giving evidence to the fact that electromagnetic fields having energy density of <10 mW/cm^2 within the range of wave lengths λ = 1–10 mm are able to influence accumulation of microorganism biomass [2–4], synthesis and strength of enzymes [5–7], as well as transport of ions K^+ and H^+ through cellular membrane [8]. All the above mentioned effects are potentially promising in terms of biotechnological processes optimization [8,9]. However, more profound understanding of their mechanisms is essential.

The general mechanism of biological effects of millimeter radiation is not clear yet [10]. Currently, there are three conceptions concerning the mechanism of low intensity millimeter EMR influence; however, these do not take into account chemical phenomena occurring on the surfaces of polymers. The first conception explaining the mechanism of millimeter radiation effects is based on the assumption that electromagnetic fields (EMF) participate in informational processes inside an organism. The following factors constitute the basis for the hypothesis: first, quantum energy of microwave radiation is smaller than the energy of the heat motion of molecules; second, quantum energy of millimeter radiation at λ = 1–10 mm is 0,124–1, 24 meV, which is by two or three orders of magnitude smaller than the energy of hydrogen binding (~0,2 eV); therefore, to break it, a very powerful radiation flow is necessary, which would lead to a significant increase in the temperature of the system. And the change of a particular biological parameter (e.g., some specific enzyme activity) after the microorganism being exposed to millimeter radiation only appears in narrow frequency bands, which often make 10^{-3}–10^{-4} of the average frequency, i.e. so called sharp resonance effect takes place [11–13].

The second conception is based on the fact that EMF acts at the genetic level [8,14–18]. Some authors believe that EMF effects on the genetic structure of a cell are related to indirect effects of its local heating as well as to free radicals formation or interaction with DNA reparation mechanisms.

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The third conception states that millimeter-range EMF influences transportation of ions and protons through cellular membrane and energy conversion [8,19–22].

To testify the hypotheses suggested, it is necessary to study the primary event of EMF energy quanta absorption by living systems and their surrounding media. As is well-known, under the influence of millimeter-range EMF in distilled water, electronic configuration of molecules changes and so does the intermolecular structure of H_2O, which can remain in this state for several days [23,24]. It should be noted that in most scientific works not enough attention is paid to the existence of the adsorbed phase of water molecules (hydrated shell) and processes of its phase transitions on hydrophilic surfaces of biopolymers [25–30]. The processes of growth or degradation of the adsorbed phase on hydrophilic surfaces of biopolymers play the key role when electromagnetic radiation affects water-bearing systems [31,32].

Due to the fact that in experiments EMRs of different frequencies and different models of biological objects were used, comparing the data available is fairly difficult. Thus, conducting complex researches is of great current interest, those aimed at understanding the primary molecular processes of millimeter EMR effects on living bacteria including studying the effects in nutrient medium and the impact on growth characteristics of a culture.

2. Materials and methods

2.1. Test organism

The sensor bioluminescence system "Ekolyum-8" (*E. coli* K12 TG1 with clone *luxCDABE* genes *P. luminescens* ZM 1) was used as the research object. The strain was obtained and deposited in the Laboratory of Biologically Active Substances at the Department of Microbiology of the Faculty of Biology, Lomonosov Moscow State University. For the strain cultivation a reconstituted solution "Nutrient Broth for Microorganisms Cultivation, Dry Fish Meal Hydrolysate" (FMH-broth) was used (Pharmacopeia article 42-3378-97) (State Research Center for Applied Microbiology and Biotechnology, Russia) (Pharmacopeia article 42-3378-97).

2.2. Samples preparation

Preliminary work for conducting measurements involved rehydration and preparation of working dilution of the lyophilize indicator strain *E. coli* with the help of bacteria-free water. The diluted indicator culture was allowed to stay minimum 30 min at temperature $(22 \pm 2)\,^{\circ}C$.

To carry out *atomic-force microscopy* (AFM), the culture was preliminarily purified from the components of nutrient and protective (to against lyophilization) media with the help of fourfold centrifugation. The purified and reconstituted culture was bottled into polymer test-tubes and subjected to SHF-irradiation. After being processed, suspension of microorganisms of 10 mcl in volume was placed onto the object-plate. With the help of a centrifuge SPIN12000 (Midas), the test-strain culture was spread over the plate and put into the camera of an atomic-force microscope.

To *study the growth,* 0.5 ml (10^7 CFU/ml) of the rehydrated culture was inoculated into each of three test-tubes containing 5 ml of FMH-broth.

2.3. Experimental apparatus for samples irradiation

The experimental apparatus for samples irradiation at the frequency of 37.01 GHz is presented in Fig. 1. The source of microwave radiation was generator G4-156 on the Gunn-effect diode 1, which provides plane-polarized radiation with the capacity of 20 mW in the continuous wave generation mode. Microwave signal from the generator, passing through insulating ferrite isolator 2, attenuator 3 and matching E-H transformer 4, was transferred to the microwave horn antenna 5 with the rectangular aperture of 72×34 mm. The lens 6 formed the beam of microwave radiation and directed it to the polymer (polyethylene) round test-tube containing the sample; the tube was placed firmly in the 2 styrofoam stand 7 transparent for microwave radiation. The dielectric lens 6 formed a two-dimensional parallel beam of microwave radiation (wave H_{10}). The electric field vector $\vec{E}$ was directed vertically. The microwave beam section at the half-power level and the test-tube with the sample in it are presented on the left of Fig. 1. Total power loses included loses that occurred in the waveguide transmission line, dissipation beyond the section at the half-power level, and reflection from the vessel exposed to irradiation; all these made up about 30% of the power generated. Thus, microwave radiation power flow effecting the sample under investigation was about 0.4 mW/cm^2. The distance from the antenna aperture to the center of the tube was 38 cm, which provided work in the far field of the microwave radiation antenna with lens.

2.4. Spectrophotometric analysis

Spectra of FMH-broth (fish-meal hydrolysate) optical density were recorded in the range of 200–1400 nm by the spectrophotometer "UV-2600" (SHIMADZU, Japan). Distilled water served as reference material.

2.5. NMR relaxometry

The study of NMR relaxometry parameters was performed with the use of the relaxometer "mq10 NMRAnalyzer" (Bruker, USA). According to the operation manual, the temperature of the samples measured must be 40 °C, that is why the samples were thermostated during 30 min.

2.6. Atomic-force microscopy

Atomic-force microscopy was conducted with the use of the microscope "Integra Prima – NanoLaboratoriya" (NT-MDT, Russia). Scanning was performed by the constant force contact method with the use of the cantilever CSGiO, whose hardness is 0.03 N/m and radius is $L = 10$ nm. While performing the atomic-force microscopy, adhesion force $F_a = k \times \Delta Z$ was measured, where k is the cantilever's hardness, ΔZ is the difference of the cantilever's height at the moment of contact with the sample and that of its separation from the sample.

2.7. Luminometry

Identification of bioluminescence under the influence of EMF towards enterobacteria was carried out with the help of an express-test on the basis of the sensor system "Ekolyum-8". The level of the indicator culture luminosity blanking (stimulating) was determined by the luminometer "Biotoks-10" (NERA-S, Russia) in fixed intervals after irradiation: 10 min, 1–5 and 24 h. Quantitative estimation of the test-reaction parameter was presented in the numerical form (I_{il}), which matches the percentage of the increase or decrease in the indicator strain luminous intensity. The criterion of the inhibiting/stimulating effect is the change in the value of the test-object bioluminous intensity in the sample tested compared to a blank sample not exposed to irradiation.

2.8. Turbidimetry

Turbidimetry was carried out by the optical device "Densi-La-Meter II" (Erba Rus, Russia).