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EFFECT OF RADIOFREQUENCY ELECTROMAGNETIC RADIATION ON *PHOTOBACTERIUM PHOSPHOREUM LUMINESCENCE*

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Background: *The technological progress has led to the widespread use of various radiofrequency electromagnetic radiation (RF-EMR) sources. Luminous bacteria were used as test objects for research of radio waves influence on living organisms.* ***Objective:*** *The presented study was focused on the processes related to Photobacterium phosphoreum luminescence under RF-EMR: some physiological, biochemical consequences and the variations in luxb gene expression.* ***Material and Methods:*** *The IMV B-7071 strain of the luminous marine bacterium P. phosphoreum from the culture collection of the D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine was used as an object of the study. We used "UHF-62", "Ray-11" and "MRTA-02" commercial devices as a source of RF-EMR.* ***Results:*** *It has been revealed that RF-EMR affected luminescence intensity, transcriptional activity of luciferase encoding gene, superoxide dismutase activity, cell survival rate. It was found that inhibition or stimulation of P. phosphoreum IMV B-7071 luminescence intensity depends on exposure duration.* ***Conclusion:*** *The data indicated the stressful nature of the RF-EMR action. Results obtained in this study suggest that luminescence intensity of P. phosphoreum IMV B-7071 bacterial cells is an indicator of the RF-EMR biotropic impact.*

Keywords: bioluminescence, electromagnetic radiation, luxb gene expression, Photobacterium phosphoreum.

Bacterial bioluminescence is a reaction of luminous bacteria that involves a luciferase-catalyzed oxidation caused by action of an enzyme called bacterial luciferase, encoded by *lux* gene [1]. Bioluminescence intensity of luminous bacteria is an integral parameter of their metabolism which makes this phenomenon very attractive for use as a potentially very sensitive indicator of changes in the environment and the presence of toxic pollutants [2, 3]. The advantages of the bioluminescent assays are high sensitivity, short response time and easy instrumental record [4, 5].

Luminous bacteria have been used as bio-indicators since the 1950s [6]. There are express methods of quantitative toxicity determination based on measuring a decrease in luminescence intensity of bacteria after addition of toxic compounds into water samples [7–11]. One of the perspective directions of bioluminescent analysis expansion is its use to assess the degree of biological action of radiofrequency electromagnetic radiation (RF-EMR) [12, 13].

The relevance of this problem is growing due to the constant increase in the number and diversity of RF-EMR sources which leads to almost overall RF-EMR exposure on living organisms.

Objective. The RF-EMR norms in all countries are based on the results of measurements by technical means of physical characteristics and takes into account only acute thermal effects [14]. Adequate impact assessment requires quantitative methods for detecting biological effects of RF-EMR, which are complicated by the lack of appropriate assessment methods directly involving biological objects.

Simple, highly sensitive biological test systems that provide reproducibility of the results and are suitable for mass analyzes are required. Such systems include bacterial luminescent test systems that are already being used to evaluate the toxicity of various chemicals [15].

Little is known about the extent of RF-EMR influence on bacterial bioluminescence which can be essential in development of technologies based on the use of luminous bacteria as biological indicators of environmental pollutants

The aim of this paper was to report the observed effect of different RF-EMR range on *Photobacterium phosphoreum* IMV B-7071 luminescence and detect consequence on luciferase encoding gene expression, cell survival rate and superoxide dismutase activity.

Materials and Methods. *Bacteria, culture conditions.* The IMV B-7071 strain of the luminous marine bacterium *P. phosphoreum* from the culture collection of the D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine was used as object of the study. Bacteria species identification was confirmed by the sequencing of 16S rRNA gene region. The nucleotide sequence was submitted to the GenBank nucleotide sequence database (http://www.ncbi.nlm.nih.gov/genbank) under accession number KF656787.

Bacterial biomass was grown for 8 hours in liquid medium composed of (g/l): peptone – 5.0; yeast extract – 1.0; NaCl – 30.0; Na_2HPO_4 – 5.3; KH_2PO_4 x $2H_2O$ – 2.1; $(NH_4)_2HPO_4$ – 0.5; $MgSO_4$ x H_2O – 0.1; glycerol – 3.0 ml x L^{-1} [16] in 750 ml flasks with 100 ml of medium at 145 rpm and 22˚C. The bacterial suspension of the same volume and concentration (V = 1 ml, 10^7 cells/ml) was exposed to irradiation from different RF-EMR sources.

Exposure to RF-EMR. We used “UHF-62” (#3201, Russian Federation), “Ray-11” (Medical Equipment Factory, Russian Federation) and “MRTA-02” (#00533, “Radmir”, Ukraine) commercial devices as a source of RF-EMR. The characteristics and irradiation parameters of these devices are listed in Table 1. The distance between emissivity antenna and object of influence was 5 cm. RF-EMR effect was estimated by the change in the intensity of luminescence. The control tests were carried out in the same conditions without irradiation. The exposure duration of 5 and 15 min was chosen as the most influencing on bacteria luminescence changes.

Table 1

Technical characteristics of RF-EMR sources

RF-EMR source	Power Output, W	Generator operating frequency, MHz	RF-EMR range
UHF -62	15	40.68	Very high frequency (VHF EMR)
RAY -11	15	2450	Ultrahigh frequency (UHF EMR)
MRTA-02	10^{-4}	57000-62500	Extremely high frequency (EHF EMR)

The temperature factor was used as an additional control to evaluate possible thermal effect of RF-EMR. For this purpose bacterial cell suspension was heated up to 42.0˚C (temperature setting with an accuracy of 0.1˚C) with portable thermostat TDB-120 (Biosan, Latvia).

Measuring of bacteria luminescence. Experimental luminometer based on photomultiplier tube (FEU-115M, 1400 V) was used to register the bioluminescence intensity. The luminescence intensity changes of the sample were described as bioluminescence index – BI [5]. BI was calculated as a ratio of test sample luminescence intensity (I_t) to the control sample luminescence intensity (I_c):

$$BI = I_t/I_c.$$

Genetic analysis and SOD activity evaluation. RNA isolation, cDNA synthesis and qRT-PCR were performed as it was described in [17]. Relative *luxb* gene expression in exposed samples compare to control one was calculated using $2^{-\Delta\Delta Ct}$ method [18], 16S rRNA gene was used as endogenous reference [17].

Antioxidant activity of the tested bacteria was studied for key antioxidant defence enzyme superoxide dismutase (SOD). SOD activity was evaluated by *in vitro* inhibition of epinephrine autoxidation [19]. Antioxidant activity of tested bacteria was expressed as percentage of epinephrine autoxidation inhibition. SOD activity was detected in cell-free extracts using spectrophotometry and calculated as the % relatively to the total protein concentration in the sample. Total protein concentration was measured using Lowry method [20].

The experiments were conducted in triplicate. Statistical data processing was carried out with STATISTICA program, version 13 (http://statsoft.ru/).

Results. *RF-EMR effects on bacterial luminescence.* To assess influence of RF-EMR on *Photobacterium phosphoreum,* luminescence intensity was analyzed after irradiation (under different RF-EMR range). Bacterial cells were exposed to VHF EMR, UHF EMR (15 W power) and EHF EMR (100 μW power) for 15 min. The results of RF-EMR impact on bacteria luminescence showed the luminescence intensity dependence on the power and exposure duration (Fig. 1).

Exposure with 15 W power (VHF and UHF EMR) led to nonlinear dependence between the luminescence intensity and the exposure duration. Luminescence intensity analysis of 5 min 15 W power VHF EMR irradiation revealed an increase in the intensity of luminescence by 22% in comparison with control. The 15 min treatment with VHF EMR resulted in significant decrease up to 68% of bacteria luminescence intensity in comparison with control.

The similar tendency was observed after UHF exposure. 5 min 15 W power UHF EMR influence on bacterial cells led to an increase in luminescence intensity by 45% in comparison with control. Decrease in bioluminescence by 67% in comparison with control was observed after 15 min from the start of UHF EMR irradiation.

An increase in luminescence intensity during 15 min of irradiation time was noted after EHF EMR irradiation exposure with frequency and 100 μW power on bacterial cells.

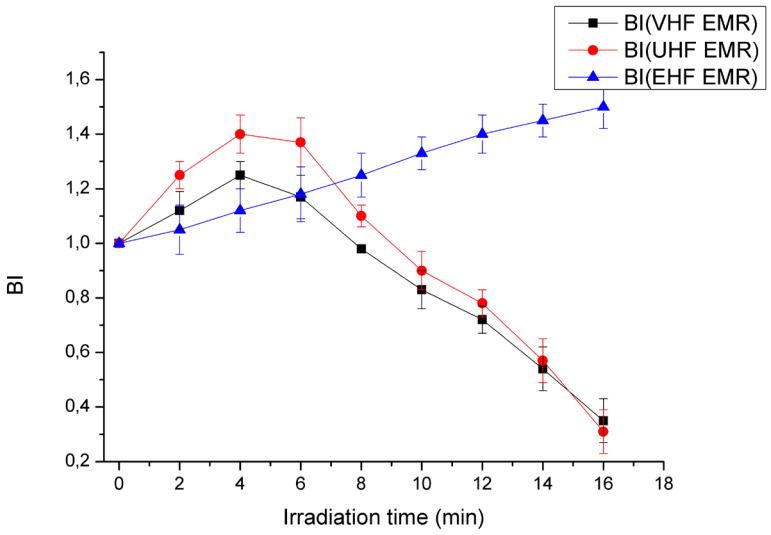

Fig. 1. RF-EMR influence on bacterial luminescence

The marked dependence demonstrated the effects of stimulation and inhibition of luminescence intensity under RF-EMR influence on bacteria. We observed an increase in the intensity of bacterial luminescence after 5 min of exposure for all types of radiation. While 15 min exposure in the case of 15 W power VHF EMR and UHF EMR resulted in a significant decrease in the luminescence intensity.

These data demonstrate significant changes in the luminescence intensity under the action of 15 W power due to heating of the medium.

Since the most pronounced effects were observed during the 5 and 15 min action of EMR exposure, further studies were focused on these values of the exposure duration.

Influence of medium temperature on bacteria luminescence. The mechanism of high-intensity RF-EMR action was primarily compared with the influence of temperature [21]. In this connection special attention was paid to the influence of the temperature on luminescence intensity.

As we have shown previously for *P. phosphoreum* IMV B-7071, the maximum level of luminescence was in the range of 10 to 28˚C [22]. At higher temperatures a sharp decrease in bioluminescence was detected.

Temperature elevation of bacterial suspension, which was dependent on the power and duration of electromagnetic radiation exposure, was observed during irradiation by various EMR sources (Fig. 2).

Nonspecific effect of the different types RF-EMR impact is in the object temperature elevation, which is dependent on the emission power. The temperature rise was observed when using 15 W VHF EMR and UHF EMR power, but no temperature changes were detected after 100 μW EHF EMR exposure.

Analysis of temperature change after 15 W power UHF EMR irradiation showed that an increase in medium temperature was higher in all tested samples compared to the VHF EMR (Fig. 2). The temperature detected in bacterial culture after 5 min UHF EMR irradiation was 29˚C and luminescence intensity

was increased by 22%, but for VHF EMR opposite effects were observed. After VHF EMR irradiation at the same temperature the luminescence intensity decreased by 30%. Thus, the same temperature values for the different action induced opposite effects for VHF EMR and UHF EMR.

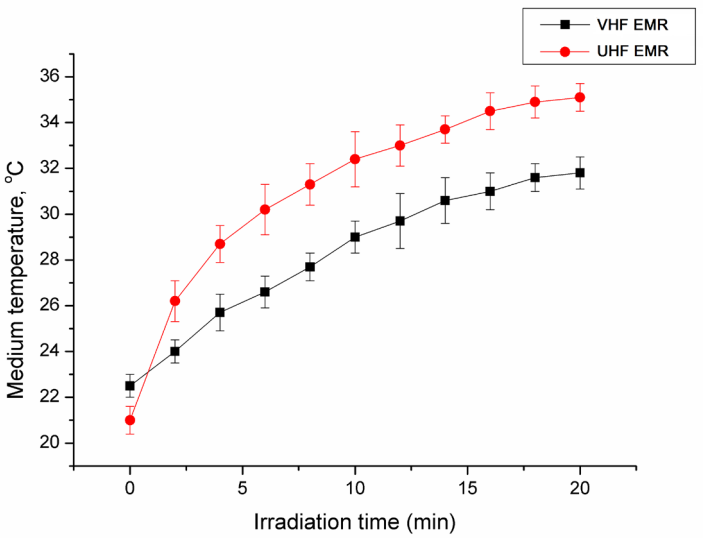

Fig. 2. Temperature changes in bacterial suspension after RF-EMR irradiation

This temperature-related difference between the values of luminescence intensity of bacteria irradiated with different ranges of RF-EMR allows to suggest that the changes of luminescence intensity caused by RF-EMR might have thermal and non-thermal components.

Data obtained for low-intensity EHF EMR exposure at the 61220 MHz frequency with an average power of 100 μW, when the stimulation of bacterial luminescence was observed, can be a proof of a possible non-thermal mechanism of RF-EMR action. In this case, irradiation did not cause a change in the temperature of the culture medium because of the low absorbed power.

It is also important to note that the RF-EMR effect may be due to the formation of free radical oxidation products [23]. Same activation of the antioxidant system might be a proof of this assumption. However the exposure of bacterial cells to RF-EMR irradiation caused very small increase of SOD activity (up to 0.45 %/mg).

Cell survival rate. It is important to note that all radiation modes were accompanied by a reduction in *P. phosphoreum* IMV B-7071 cell viability. If in the case of 15 W irradiation the fraction of surviving cells was 32 ± 9%, then after 100 μW power EMR exposure it increased up to 68 ± 12% being still lower than control (Fig.3). The data on *P. phosphoreum* IMV B-7071 cell survival under irradiation indicated the stress of RF-EMR action even at absorbed low energy radiation.

Expression of luxb gene under different type of EMR. It is known that luminescence, as the peculiarity of luminescent bacteria, is caused by activity of luciferase enzyme consisting of two main subunits [24]. In our study *luxb* gene expression level was evaluated under three types of EMR.

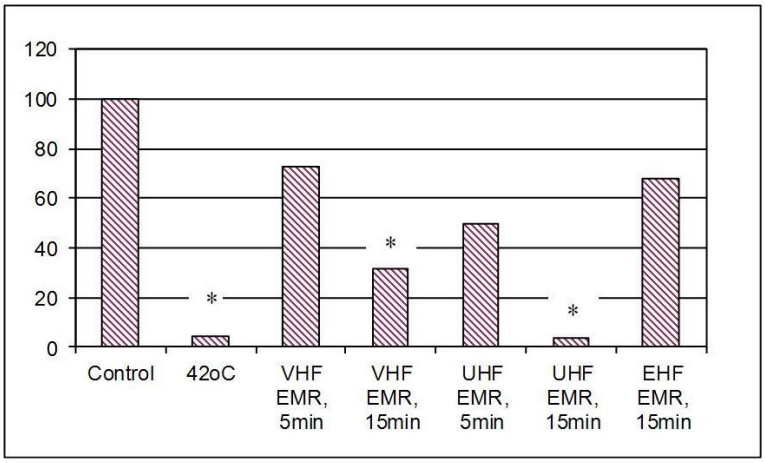

Fig. 3. Cell survival rate in bacterial suspension after RF-EMR irradiation. * – $p<0.05$

Results of *luxb* gene expression analysis showed the increase of gene activity value in 3.8 times after 15 min exposure to EHF EMR (Fig.4 B), in 3.5 and 3 times after exposure to UHF EMR and VHF EMR for 5 min. 15 min exposure to the last two types of RF-EMR led to increase in *luxb* mRNA amount after UHF EMR (in 2 times) and did not cause any changes were detected after VHF EMR in comparison with non-irradiated bacteria. Besides, the dependence between gene activity and exposure duration was observed. Analysis of changes in *luxb* mRNA abundance within 15 min UHF EMR and VHF EMR exposure revealed that gene expression was decreasing during this time (Fig.4 B). The similar results were obtained for both type of EMR: increasing in *luxb* transcriptional activity after 5 min and decreasing after 15 min of irradiation. The level of expression decreased in 1.75 and 4.2 times (UHF EMR and VHF EMR, respectively) compare to samples after 5 min of exposure.

Since *luxb* gene encodes luciferase subunit responsible for light emission, we compared bioluminescence and gene expression indices under RF-EMR. The results showed correlation ($r = 0.797$) between these parameters: the higher is gene activity, the bigger is bioluminescent index. But it should be noted that the ratio between BI and expression level depends on the type of RF-EMR (Fig.4 A). For example, when the BI of 5 min 15 W VHF EMR irradiation is equal to 1.22, the *luxb* gene activity value rise in 3 times. When 5 min UHF EMR treatment resulted in BI equal to 1.45, the *luxb* gene activity value rise in 3.5 times.

Discussion. The results presented in this paper indicate that *P. phosphoreum* IMV B-7071 was highly sensitive to the action of a wide range of non-ionizing RF-EMR that appeared to change the intensity of its luminescence.

Inhibition or stimulation of the observed luminescence intensity of bacteria may indicate variability of the RF-EMR exposure-related effects. Thermal mechanism dominates in cases of RF-EMR energy absorption for more than 15 min that was manifested in the emission intensity characteristic decrease similar to the changes caused by temperature. When duration of RF-EMR exposure was less than 5 min, bacterial suspension showed the effects suggesting the existence of nonthermal nature luminescence stimulation mechanism.

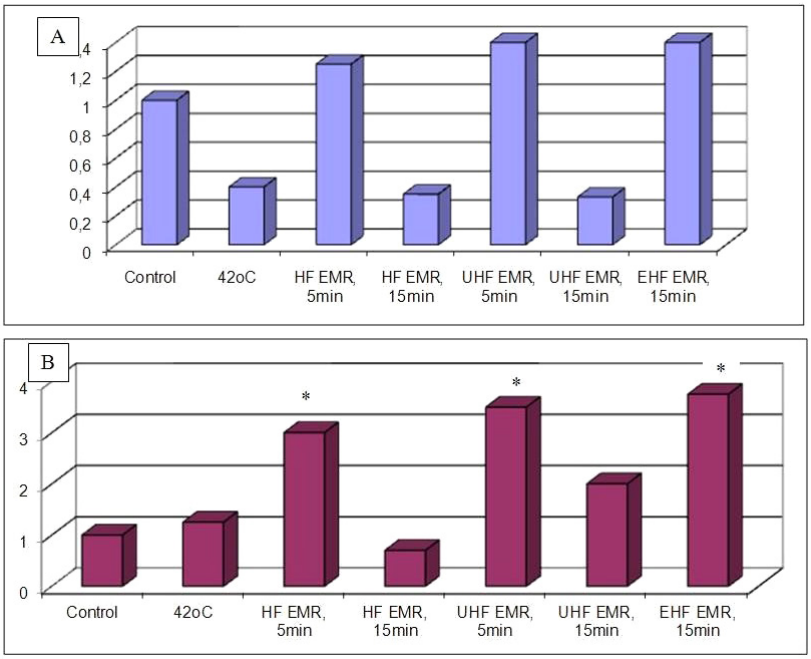

Fig. 4. *P. phosphoreum* luminescence intensity (A) and *luxb* gene expression (B) after various range RF-EMR irradiation. * – p<0.05

The data on *P. phosphoreum* IMV B-7071 cell survival rate at various regimes of the irradiation indicated a decrease in the viability of bacteria in the case of high-intensity and low-intensity RF-EMR exposure. These results suggested the stressful nature of the RF-EMR action even at low energy absorbed radiation.

Analysis of *luxb* gene transcriptional activity revealed an increase in the level of its expression after all studied types of radiation. It should be noted that in cases of bacterial luminescence stimulation after irradiation, the level of *luxb* expression exceeded control more than 3 times. Inhibition of bacterial luminescence after irradiation was also accompanied with a decrease in the expression of *luxb* gene by 1.75 times, but it was still higher than expression value in the control samples. Given that this was accompanied with a significant decrease in cell viability and the continuous increase in the level of specific gene expression, we can make conclusion about specific luminescence magnification upon EMR irradiation. In other words, under such conditions remained intact cells started to increase light emission even after significant decrease in cell viability. This phenomenon known as "quorum sensing" and associated with the change in the level of cells bioluminescence depending on the density of population, was detected for the first time in luminescent bacteria *Vibrio fischeri* [24]. This process has been well studied for this microorganism and investigated on other biological systems. In contrast to the results obtained by Tanet L. et al (2019) [25] in our case, we seem to observe the effect of communication and interaction between cells in a stressful environment. Autoregulation mechanism of cells emission intensity under stress conditions affecting the expression of lux-operon identification of regulatory biomolecules requires further study.

According to our results the relationship between SOD as a key enzyme of the antioxidant system and luminescence and electromagnetic irradiation was not detected.

A possible mechanism for the genetic control of bioluminescence under irradiation cell RF-EMR can also be association of lux-gene with DNA repair system [26]. In the case of DNA damage presence, SOS-regulon gene regulation occurs with the appropriate inclusion of DNA repair mechanisms. This mechanism of lux-genes transcription activity changes can explain the marked increase in the level of bacterial cells bioluminescence after low density UV irradiation [27].

The data obtained from the impact of organic solvents on the luminous bacteria demonstrated effects similar to EMR exposure [28]. According to this study, the degree of stimulation or inhibition of luminescence intensity of bacteria depends on both the concentration and the nature of the organic solvent, which is due to its influence on the structure of luciferase enzyme. Stimulation of luminescence was observed after the action of low concentration of solvent as in the case of short exposure. In the presence of high solvent concentration, the effect was similar to the effects of the large doses of EMR leading to the inhibition of bacterial luminescence. Perhaps this is due to the electromagnetic nature of interaction between the luciferase enzyme and one of the reaction substrates – aldehyde. The identification of mechanisms requires more extensive studies.

Thus, as shown in our study, luminescence intensity of *P. phosphoreum* IMV B-7071 bacterial cells is an indicator of the RF-EMR biotropic impact that can be used to create biosensor device for biological evaluation of non-ionizing electromagnetic radiation.

ВПЛИВ РАДІОЧАСТОТНОГО ЕЛЕКТРОМАГНІТНОГО ВИПРОМІНЮВАННЯ НА ЛЮМІНЕСЦЕНЦІЮ *PHOTOBACTERIUM PHOSPHOREUM*

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Резюме

Технологічний прогрес призвів до широкого використання різних джерел радіочастотного електромагнітного випромінювання (РЧ-ЕМВ). Як тестові об'єкти для дослідження впливу радіохвиль на живі організми нами були використані люмінесцентні бактерії. **Мета.** У представленому дослідженні було зосереджено увагу на процесах, пов'язаних з люмінесценцією *Photobacterium phosphoreum* в умовах дії РЧ-ЕМВ: деякі фізіологічні, біохімічні наслідки цього впливу та зміну рівня експресії гену *luxb*. **Матеріали і методи.** Об'єктом дослідження був штам люмінесцентних морських бактерій *P. phosphoreum* ІМВ В-7071 з Української колекції мікроорганізмів Інституту мікробіології і вірусології ім. Д.К. Заболотного НАН України. Як джерела РЧ-ЕМВ були використані комерційні пристрої – апарат «УВЧ-62»; «Луч-11», «MRTA-02». **Результати.** Виявлено, що РЧ-ЕМВ впливає на інтен-

сивність люмінесценції, транскрипційну активність гену люциферази, активність супероксиддисмутази, виживаність клітин. Інгібування або стимуляція інтенсивності люмінесценції *P. phosphoreum* IMB B-7071 залежала від тривалості впливу. **Висновки.** Дані вказують на стресовий характер дії РЧ-ЕМВ. Результати, отримані в цьому дослідженні, свідчать про те, що інтенсивність люмінесценції бактеріальних клітин *P. phosphoreum* IMB B-7071 є показником біотропного впливу РЧ-ЕМВ.

Ключові слова: біолюмінесценція, електромагнітне випромінювання, експресія гену *luxb*, *Photobacterium phosphoreum.*

ВЛИЯНИЕ РАДИОЧАСТОТНОГО ЭЛЕКТРОМАГНИТНОГО ИЗЛУЧЕНИЯ НА ЛЮМИНЕСЦЕНЦИЮ *PHOTOBACTERIUM PHOSPHOREUM*

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Резюме

Технологический прогресс привел к широкому использованию различных источников радиочастотного электромагнитного излучения (РЧ-ЭМИ). Как тестовые объекты для исследования влияния радиоволн на живые организмы нами были использованы люминесцентные бактерии. **Цель.** В представленном исследовании было сосредоточено внимание на процессах, связанных с люминесценцией *Photobacterium phosphoreum* в условиях воздействия РЧ-ЭМИ: некоторые физиологические, биохимические последствия этого влияния и изменение уровня экспрессии гена *luxb*. **Материалы и методы.** Объектом исследования был штамм люминесцентных морских бактерий *P. phosphoreum* ИМВ В-7071 из Украинской коллекции микроорганизмов Института микробиологии и вирусологии им. Д.К. Заболотного НАН Украины. В качестве источников РЧ-ЭМИ были использованы коммерческие устройства: аппарат «УВЧ-62»; «Луч-11», «MRTA-02». **Результаты.** Выявлено, что РЧ-ЭМИ влияет на интенсивность люминесценции, транскрипционную активность гена люциферазы, активность супероксиддисмутазы, выживаемость клеток. Ингибирование или стимуляция интенсивности люминесценции *P. phosphoreum* IMB B-7071 зависела от продолжительности воздействия. **Выводы.** Данные указывают на стрессовый характер воздействия РЧ-ЭМИ. Результаты, полученные в этом исследовании, свидетельствуют о том, что интенсивность люминесценции бактериальных клеток *P. phosphoreum* ИМВ В-7071 является показателем биотропного влияния РЧ-ЭМИ.

Ключевые слова: биолюминесценция, электромагнитное излучение, экспрессия гена *luxb, Photobacterium phosphoreum.*

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