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## EVALUATION OF MAGNETOSENSITIVITY OF *PHOTOBACTERIUM PHOSPHOREUM*

**Background.** Currently, research is being conducted to identify the mechanisms that enable living organisms to sense and utilize the Earth's magnetic field for orientation and navigation. The primary hypothetical mechanisms under active discussion include the radical pair model, which involves magnetosensitive free radical redox reactions in enzymatic systems containing oxygen molecules and flavin compounds (such as cryptochromes and bacterial luciferases), as well as the model involving intracellular magnetic magnetite particles interacting with the magnetic field. Our focus is on the first hypothesis. So, the purpose of our study was to find out the features of the influence of a static (SMF) and extremely low frequency ELF MF magnetic field on the bacterial luminescence of *P. phosphoreum*. Notably, photobacteria are widely used as bioindicators of water pollution and indicators of exposure to various biologically active compounds.

**Methods.** We measured the bioluminescence of *P. phosphoreum* in liquid media at room temperature (22–24 °C). The baseline bioluminescence was evaluated over several days following inoculation in the culture medium. Bioluminescence was recorded using digital photoregistration, with subsequent image processing conducted in ImageJ or OriginPro. Magnetic field exposure was applied in two modes. In the first mode, bacterial suspensions were exposed to the magnetic field continuously from the moment of inoculation throughout the entire growth period. In the second mode, short-term magnetic field exposure was applied for several minutes after active hydrodynamic stirring of the bacterial suspension, which triggered a burst of luminescence, followed by fading and return to the baseline level. The magnetic field induction was measured using a Hall sensor.

**Results.** Relatively strong static magnetic fields in the range of 2–8 mT weakly activated bioluminescence during the active growth phase (log phase) of the bacterial population, but they statistically significantly suppressed the glow of bacteria during their maximum luminescence and subsequent dimming. The magnitude of the effects of the magnetic field was small, approximately 15 % relative to the control values. The influence of a low-frequency magnetic field with a frequency of 7.85 Hz and induction of 100 μT stimulated the baseline bioluminescence of the photobacteria. At the same time, the magnetic field did not significantly affect either the concentration of oxygen or the concentration of bacterial cells in suspension, indicating a direct influence of magnetic fields on the metabolic processes associated with the bioluminescent system of bacterial cells. The short-term exposure of extremely low frequency magnetic field resulted in slow but statistically significant increase in the intensity of baseline bioluminescence by 5-10 % after activation of glow by the hydrodynamic stirring of the bacterial suspension.

**Conclusions.** *P. phosphoreum* is sensitive to the action of static and extremely low frequency fields, showing efficiency of MF-influence within 15 % of the control values. A peculiarity of the effect of static magnetic fields on bacterial luminescence is the dependence of the effects on the induction of the magnetic field and the phase of development of the bacterial populations' glow. During the luminescence growth phase, SMF-activation was observed, while in the luminescence fading phase, suppression occurred. A magnetic field of extremely low frequency (7.85 Hz) with 25 and 100 μT activated bacterial luminescence in different MF exposure modes. This bacterial model of magnetosensitivity is convenient for further experimental verification of the hypothesis regarding the magnetosensitivity of radical pairs.

**Keywords:** bacterial bioluminescence, magnetic field influence, biological magnetosensitivity, *Photobacterium phosphoreum*.

### Background

In recent years, substantial research has been dedicated to understanding the mechanisms by which living organisms detect and utilize the Earth's magnetic field for spatial orientation and navigation. This ability, often referred to as magnetoreception, is believed to be governed by two primary hypothetical mechanisms. The first is the radical pair mechanism, which proposes that magnetosensitive redox reactions involving free radicals occur in enzymatic systems. These systems typically contain oxygen molecules and flavin compounds, such as cryptochromes and bacterial luciferases. Cryptochromes, in particular, have been identified as photoreceptive proteins that can form magnetically sensitive radical pairs in the presence of light, potentially allowing organisms to detect

geomagnetic fields. Recent studies have further elucidated the role of cryptochrome-based radical pairs in magnetoreception in animals and explored how external magnetic fields influence these reactions (Hore, & Mouritsen, 2016; Kavet, & Brain, 2021).

Another significant hypothesis involves magnetite-based magnetoreception, wherein intracellular magnetic particles of magnetite (Fe<sub>3</sub>O<sub>4</sub>) within certain cells interact with geomagnetic fields to provide directional cues. These magnetite particles, thought to be contained within specialized receptors in organisms, respond to the magnetic field's direction and strength, contributing to navigational behaviors observed in animals such as migratory birds and marine species (Kirschvink, Walker, & Diebel, 2001; Shaw et al., 2015).

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In our study, we focused on the radical pair mechanism and specifically investigated the effects of constant and extremely low frequency magnetic fields on the bioluminescence of *Photobacterium phosphoreum*. This bacterial luminescence is driven by a reaction involving the oxidation of flavins, which may be sensitive to magnetic fields due to the radical intermediates formed. *Photobacterium phosphoreum* is widely recognized as a bioindicator for environmental studies, particularly for assessing water quality and monitoring the presence of biologically active pollutants. Its bioluminescent response serves as a sensitive indicator of toxic substances in aquatic environments, providing a valuable tool for ecotoxicological assessments (Ribo, & Kaiser, 1987; Li et al., 2022).

**So, the purpose** of our study was to find out the features of the influence of a static (SMF) and extremely low frequency ELF MF magnetic field on the bacterial luminescence of *P. phosphoreum*.

**Methods**

The culture of *Photobacterium phosphoreum* IMV B-7071 was used in this study. This bacterial strain is in the depositary of the D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences NAS of Ukraine. The species identification of the bacteria 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. The photobacteria were cultivated at room temperature of 22–24°C in the liquid nutrient medium of the following composition (g/L): peptone – 5.02 yeast extract – 1.0; NaCl – 30.0; Na<sub>2</sub>HPO<sub>4</sub> – 5.3; KH<sub>2</sub>PO<sub>4</sub> x 2H<sub>2</sub>O – 2.1; (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> – 0.5; MgSO<sub>4</sub> x H<sub>2</sub>O – 0.1; glycerol – 3.0 mL/L, distilled water – up to 1 L, pH 7.0.

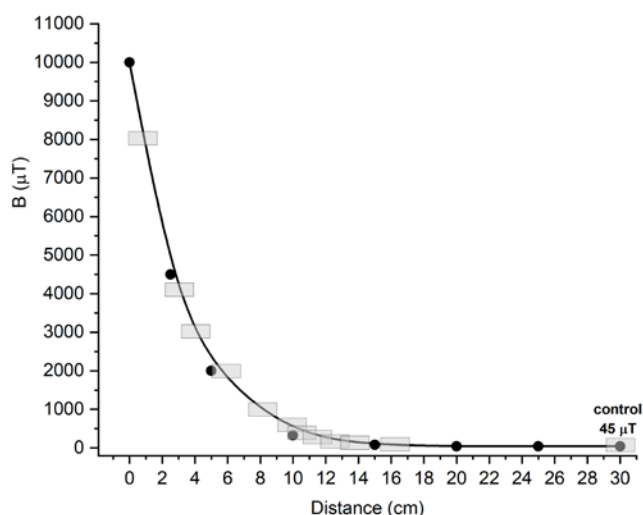
The cell concentration was measured using an optical method based on the light scattering properties of bacterial suspensions. The optical density of the suspensions was

measured using KFK-2 photocolimeter with 640 nm filter in glass cuvettes with an optical path length of 0.5 cm. Optical density value of  $D=0.1$  corresponded to a concentration of 10<sup>7</sup> cells/ml. In this study the initial cell concentration in the nutrient medium, which corresponded to the initial lag phase, for all samples was set to 10<sup>7</sup> cells/ml.

Oxygen concentration in bacterial suspensions was measured using an EZODO-7031 oximeter (GonDO Electronic, Ltd., Taiwan) based on a Clarke membrane electrode with a temperature correction function.

Bioluminescence measurements were performed in dark room using digital photo-registration of sample luminescence with Canon 700D camera set to an ISO sensitivity of 12000, an aperture of f/5.6, and an exposure time of 2 s. Baseline bioluminescence was assessed without stirring the bacterial suspension at 3, 6, 18, 24, and 30 h after the start of the experiment. Bioluminescence parameters were analyzed using OriginPro or ImageJ (NIH), a cross-platform open-source software designed for image processing and analysis in biological, medical, and other research fields. The bioluminescence intensity was recorded in arbitrary units (OriginPro) or relative brightness values in the 8-bit system (ImageJ), where each color channel (red, green, blue) or the mean of the color channels ranges from 0 to 255.

A static magnetic field (SMF) was generated using flat neodymium magnets with a diameter of 1.5 cm. Samples of bacterial suspensions were placed directly adjacent to the magnets and at specific distances from them, considering the magnetic field induction gradient. Such setup allowed for the simultaneous exposure of bacterial suspension samples to a static field with different induction values (Fig. 1). Magnetic field induction was measured using a Hall sensor paired with Gauss Meter software by Keuwlsoft. Control samples were positioned at a distance of 30 cm from the magnet, where the laboratory's ambient static magnetic field induction maintained a typical value of 45 μT.



**Fig. 1. Magnetic field gradient (solid line) and placement of bacterial suspension samples (gray rectangles)**

**Remarks :** The solid line represents the induction gradient of the static magnetic field, obtained by approximating the induction values at various distances. The point "0" indicates the center of the magnet area. The control samples are positioned 30 cm from the magnet.

Extremely low-frequency magnetic field (ELF MF) with a frequency of 7.85 Hz and induction levels of 25 μT and 100 μT was generated using Helmholtz coils. The direction of the induction vector was oriented vertically. The selection of

both the frequency and the induction of the magnetic field was based on their discovered biological activity and ecological significance. The frequency of 7.85 Hz corresponds to the primary harmonic of the Schumann

resonances, the phenomenon for which significant biological effects have been documented (Cherry, 2002). Furthermore, an induction of 100  $\mu\text{T}$  represents the maximum permissible exposure level for low frequency magnetic fields in domestic environments, as stipulated by existing hygienic standards.

A sinusoidal current was supplied to the coils from a low-frequency generator. Control of the frequency and induction of the magnetic field was carried out using a Hall sensor. Additionally, analysis of the density spectrum of the recorded signal was carried out using the AC Magnetic Field Meter software from Keuwlsoft. The average level of background low-frequency electromagnetic noise in the laboratory where the research was conducted ranged from 0.2 to 0.5  $\mu\text{T}$ , with maximum amplitudes in the frequency range of 1–20 Hz.

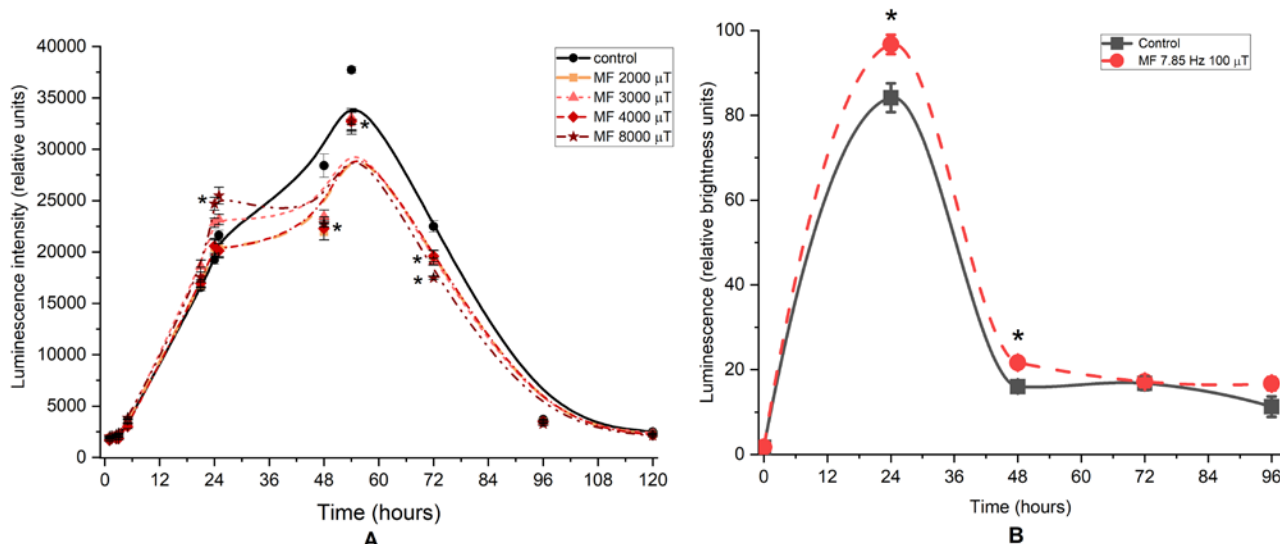
Exposure in the magnetic field was carried out in two modes. In the first mode, the exposure of bacterial suspensions in the magnetic field was carried out continuously, starting from the moment of inoculation of the bacterial culture with a density of  $10^7$  cells/ml until the end of the experiment, when the concentration of cells increased several times. In the second mode, there was a short-term exposure to a magnetic field, during which the bacterial suspensions exhibited an intense glow that developed 18–24 h after the lag phase began. For this mode ELF MF was applied from the moment of active hydrodynamic shaking, which caused a powerful flash of bioluminescence, followed by its extinction and return to the base level, after which we measured the level of bioluminescence and turned off the magnetic field. The features of hydrodynamic activation of bioluminescence and its time dynamics are described in (Gromozova et al., 2024).

Statistical data analysis was performed using the Origin Pro software platform. Average values and standard errors were calculated. The statistical significance of the difference in mean values was assessed using an unpaired t-test. The statistical sample size for each type of sample was  $n = 8$ .

**Results**

Fig. 2A shows the results of the study of the effect of SMF on the state of bacterial populations during 120 h. Relatively strong SMF in the range of 2–8 mT weakly activated bioluminescence during the active growth in log-phase of the bacterial population, but it statistically significantly suppressed the glow of bacteria during their maximum luminescence and subsequent dimming (Fig. 2A). The magnitude of the effects of the magnetic field was small, approximately 15 % relative to the control values, but statistically significant. Attention is drawn to the fact of differently directed action of SMF in different phases of bacterial population development. According to (Gromozova et al., 2024), there is an active reproduction of bacterial cells during the first or/and second day, which is accompanied by an increase of the intensity of their glow, reaching the maximum level (first phase of bioluminescence). Further growth of the density of the cell population leads to suppression of bioluminescence to its almost complete attenuation (second phase of bioluminescence).

Thus, the results of the study indicate the dependence of the direction and magnitude of the effect of the SMF on the functional state of bacterial cells. In our opinion, this fact proves that the long-term continuous influence of SMF with induction for range of several mT is realized through the mechanisms of intracellular regulation of metabolism. Probably, in this case, the hypothetical direct action of the magnetic field at the molecular level by the mechanism of recombination of radical pairs in the active center of bacterial luciferase does not play a key role for this range of magnetic field induction. Decreasing of value of SMF induction to the range of 0.5–1.0 mT led to multidirectional effects of the static magnetic field. In particular, SMF with an induction of 0.830 mT statistically reliably inhibited the luminescence of photobacteria by an average of 10–15 % in the attenuation phase of bioluminescence, while SMF with an induction of 0.670 and 1 mT stimulated the luminescence of photobacteria in range 10–15 % relatively control samples.



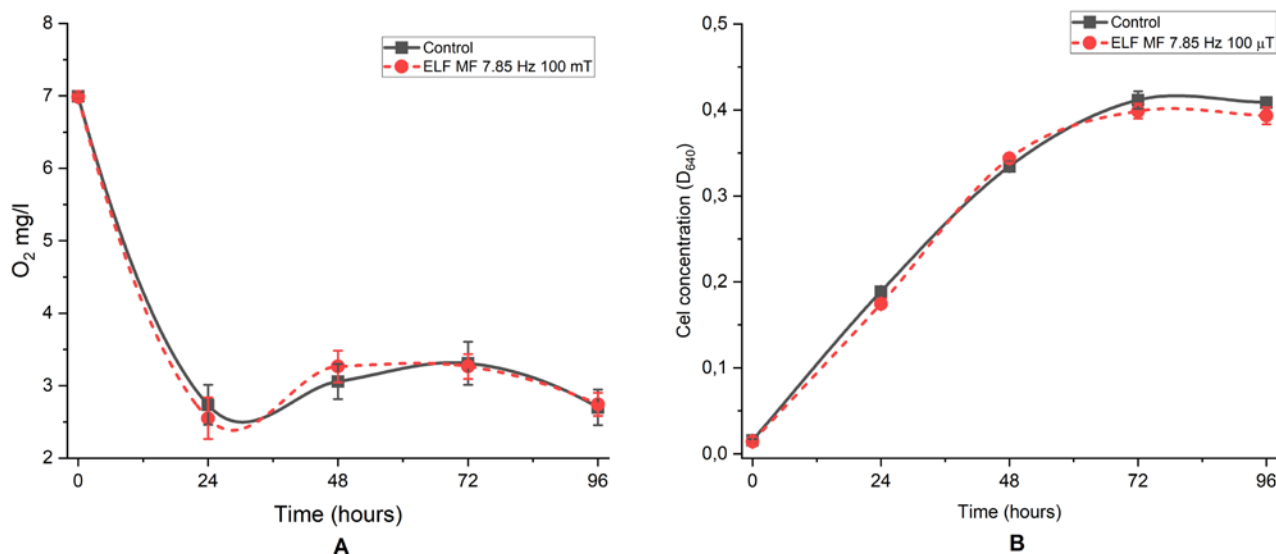
**Fig. 2. Effects of static (A) and extremely low frequency magnetic field 7.85 Hz 100  $\mu\text{T}$  (B) on basic luminescence of *P. phosphoreum***

**Remarks :** SMF and ELF MF were continuously applied during the entire period of observation of the development of the bacterial population in time (first mode of MF exposure); \* – statistically significant  $P < 0,05$ . Data processing in Fig. 2A was carried out in Origin Pro, in Fig. 2B – in ImageJ.

In the microtesla range of MF induction, the stimulating effect was observed for the level of 99–100  $\mu\text{T}$  in both phases of the development of bacterial bioluminescence. During the first day, this effect also stimulated the reproduction of photobacteria, and at the moment of 24 h, the density of the bacterial population increased statistically significantly by an average of 15–20 % ( $P < 0.05$ ). The presence of such amplitude "windows" in the influence of SMF on biological processes indirectly indicates the possible participation of the radical pair mechanism in the biological effects of this physical factor. Similar frequency-amplitude "windows" were observed in studies on models of free radical processes in liposomes (Martynyuk, & Tseyslyer, 2022). It is important to note that the maximum effects of SMF exposure were

observed at the peak of photobacterium bioluminescence and in its attenuation phase.

The long-term influence of ELF MF of 7.85 Hz and induction of 100  $\mu\text{T}$  stimulated the baseline bioluminescence of the photobacteria *P. phosphoreum* (Fig. 2B). This resulted in statistically significant increase in the intensity of baseline bioluminescence by 10–15 %. The direction of the effect of ELF MF 7.85 Hz 100  $\mu\text{T}$  is very similar to the effect of SMF with same induction. Probably, such ELF MF is perceived by bacterial cells as quasi-static, so they show similar response to its influence. It important to note that ELF MF not significantly affected on concentration of oxygen or the cell concentration in bacterial suspension (Fig. 3). Possibly, this fact indicating direct influence of magnetic fields on the several enzymatic processes closely associated with the bioluminescent system of bacterial cells.

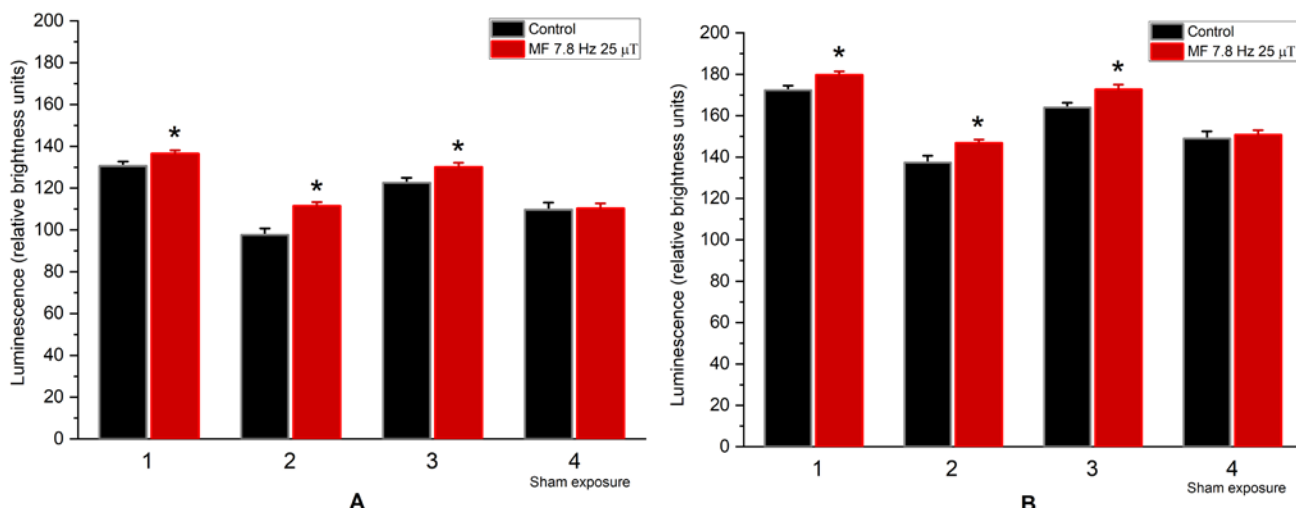


**Fig. 3. Time dynamics of oxygen concentration (A) and cell concentration (B) in bacterial suspensions in control samples and under exposure ELF MF 8 Hz 100  $\mu\text{T}$**

In experiments with long-term continuous exposure to SMP, certain changes in the intensity of bacterial luminescence were found, which are most likely associated with system changes in the metabolism of bacterial cells. However, there are still questions about the biological effectiveness of short-term effects of MF on the intensity of light of photobacteria, because significant systemic metabolic changes in cells in the entire bacterial population cannot occur during a short-term exposure. In experiments with weak ELF MF influence for short time on bacterial suspensions, the glow of photobacteria was activated by intensive mixing (hydrodynamic activation). In this mode, the ELF MF was turned on at the moment of hydrodynamic activation of bioluminescence and continued to affect the bacterial suspensions until the photobacteria glow

decreased to the background level, the intensity of which was estimated by digital photoregistration.

Fig. 4 shows the results, which demonstrate a certain variability in the basic glow of bacterial suspensions for different experimental days. Independently of this, we observed a small but statistically significant increase in the intensity of the background (baseline) bioluminescence under the influence of weak ELF MF (Fig. 4). The increase of the intensity of the glow of photobacteria upon ELF MF influence in this research mode is only 5-10 %, but this effect is reliably registered and satisfactorily reproduced. Possibly, for this mode of ELF MF influence, the radical-pair mechanism plays a key role in the biological effectiveness of this physical factor. This assumption should be carefully tested in further studies.



**Fig. 4. Effect of ELF MF 7.8 Hz 25 µT on the background (baseline) bioluminescence of *P. phosphoreum* in the green (A) and blue (B) colored channel of the digital image in three repeated experiments**

**Remarks:** The numbers of repeated experiments are given on the horizontal axis. The last experiment was carried out in sham exposure regimes, where the test samples were placed in the Helmholtz coils, but no electric current was applied to them; \* – statistically significant changes,  $P < 0.05$ .

To discuss the results, it is worth paying attention to some phenomena that are important for understanding the features of behavior and evolution of photobacterial populations over time and their response to external factors. As we found earlier (Gromozova et al., 2024), the growth of luminescence intensity reaches a certain maximum approximately in the middle of the log phase. However, as the further bacterial population density increases and reaches the stationary phase, the luminescence intensity decreases significantly until it is completely extinguished. This phenomenon is well-reproduced, including in this series of experiments, but the authors do not yet have an explanation for its nature. It is important to note that there is no mention of this behavior of photobacteria in literary sources.

The above-mentioned phenomenon plays an important role in the response of photobacteria to a stationary magnetic field. In particular, during the phase of increasing intensity of bioluminescence the influence of SMF with certain values of magnetic induction activates the glow of bacteria. But in the phase of maximum luminescence and subsequent attenuation, the influence of SMF contributes to the attenuation of bacterial luminescence. The nature of such a different response can be explained by the dependence of the response of photobacteria to the effect of SMF on their metabolic state, which changes during the transition from the log phase to the stationary phase of the development of the bacterial population. On the other hand, the magnetic field of extremely low frequency stimulates the luminescence of photobacteria under different modes of exposure, indicating different mechanisms of influence for SMF and ELF MF.

**Discussion and conclusions**

*P. phosphoreum* is sensitive to the action of static and extremely low frequency fields, showing a biological efficiency of MF within 15 % of the control values. A peculiarity of the effect of static magnetic fields on bacterial luminescence is the dependence of the effects on the induction of the magnetic field and the phase of development of the bacterial populations' glow. During the luminescence growth phase, SMF-activation was observed, while in the luminescence fading phase, suppression occurred. A magnetic field of extremely low frequency

(7.85 Hz) with 25 and 100 µT activated bacterial luminescence in different MF exposure modes. This bacterial model of magnetosensitivity is convenient for further experimental verification of the hypothesis regarding the magnetosensitivity of radical pairs.

**Authors' contribution:** Viktor Martyniuk – main idea, design and research, literature data analysis, manuscript writing, research results summarization; Yuliya Tseyslyer – conducting research, manuscript section writing, research results processing; Olena Gromozova – methodology and results discussion, literature data analysis, manuscript editing; Igor Hretskyi – experimental samples and culture media preparation, results discussion.

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## ОЦІНЮВАННЯ МАГНІТОЧУТЛИВОСТІ *PHOTOBACTERIUM PHOSPHOREUM*

**Вступ.** На цей час ведуться дослідження щодо визначення механізмів, які дозволяють живим організмам відчувати та використовувати магнітне поле Землі для орієнтації та навігації. Основними гіпотетичними механізмами, що активно обговорюються, є модель радикальних пар, яка передбачає магніточутливі окисно-відновні реакції у ферментативних системах, що містять молекули кисню та флавінові сполуки (напр., криптохроми та бактеріальні люциферази), а також модель, яка містить внутрішньоклітинні магнітні частинки магнетиту, що взаємодіють з магнітним полем. Наша увага зосереджена на першій гіпотезі. Таким чином, метою нашого дослідження було вивчення особливостей впливу постійних і наднизькочастотних магнітних полів на біоломінесценцію *Photobacterium phosphoreum*, яка базується на реакції окиснення флавінів. Варто зазначити, що фотобактерії широко використовуються як біоіндикатори забруднення води та індикатори впливу різних біологічно активних сполук.

**Методи.** Ми вимірювали біоломінесценцію *P. phosphoreum* у рідинних середовищах за кімнатної температури (22–24 °C). Базова біоломінесценція оцінювалась протягом декількох днів після інокуляції в культурне середовище. Біоломінесценцію реєстрували за допомогою цифрової фотореєстрації з подальшою обробкою зображень в ImageJ або OriginPro. Вплив магнітного поля здійснювався в двох режимах. У першому режимі бактеріальні суспензії піддавались впливу магнітного поля безперервно з моменту інокуляції протягом всього періоду росту. У другому режимі короткочасний вплив магнітного поля здійснювався протягом кількох хвилин після активного гідродинамічного перемішування бактеріальної суспензії, що викликало спалах люмінесценції, після чого вона згасала і поверталася до базового рівня. Індукція магнітного поля вимірювалась за допомогою сенсора Холла.

**Результати.** Відносно сильні постійні магнітні поля в діапазоні 2–8 мТ слабо активували біоломінесценцію під час активної фази росту (log-фази) бактеріальної популяції, проте вони статистично значущо пригнічували світіння бактерій під час їх максимальної люмінесценції та подальшого згасання. Величина ефектів магнітного поля була невеликою, приблизно 15 % від контрольних значень. Вплив низькочастотного магнітного поля із частотою 7.85 Гц та індукцією 100 мкТ стимулював базову біоломінесценцію фотобактерій. Водночас магнітне поле суттєво не впливало ні на концентрацію кисню, ні на концентрацію бактеріальних клітин у суспензії, що вказує на вплив магнітних полів на метаболічні процеси, пов'язані з біоломінесцентною системою бактеріальних клітин. Під час короткочасного впливу цього наднизькочастотного магнітного поля ми спостерігали спалах люмінесценції, ініційований активним гідродинамічним перемішуванням бактеріальної суспензії. Це призвело до повільного, але статистично значущого збільшення інтенсивності базової біоломінесценції на 5–10 %.

**Висновки.** *P. phosphoreum* чутливе до дії статичних і наднизькочастотних полів, демонструючи біологічну ефективність МП у межах 15 % порівняно з контрольними значеннями. Особливістю впливу статичних магнітних полів на люмінесценцію бактерій є залежність ефектів від індукції магнітного поля та фази розвитку світіння бактеріальних популяцій. Під час фази зростання люмінесценції спостерігалася активація СМП, тоді як у фазі згасання люмінесценції відбувалося пригнічення. Магнітне поле надзвичайно низької частоти 7,85 Гц 25 і 100 мкТ активувало бактеріальну люмінесценцію в різних режимах впливу МП. Використана бактеріальна модель зручна для подальшої експериментальної перевірки гіпотези щодо магніточутливості радикальних пар.

**Ключові слова:** бактеріальна біоломінесценція, вплив магнітного поля, біологічна магніточутливість, *Photobacterium phosphoreum*.

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