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CELL BIOPHYSICS

An Analysis of the Bioluminescence Intensity Dynamics of the Luminous Bacteria *Photobacterium phosphoreum*

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Abstract—The results of the analysis of the dynamics of the bioluminescence of the luminous bacteria *Photobacterium phosphoreum* IMB-7071 under optimal growth conditions are presented. A quasi-harmonic character of bacterial bioluminescence was revealed. The observed periods of these changes have similar values to the previously established periods of the changes in the physicochemical properties of water. Correlations between biorhythms and the quasi-harmonic character of the physicochemical properties of water are discussed.

Keywords: bioluminescence, luminous bacteria, rhythmic processes, biorhythms, water properties

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Investigations of periodic changes of the intensity of biological processes and phenomena both at the level of the functioning of a single cell [1] and of an entire organism [2–5] have been extensively described. The available literature data indicate that the temporal organization of biological systems should be characterized by an entire spectrum of periods of different durations [6]. Cosmo-physical effects, oscillations of the Earth's magnetic field, solar-activity rhythms, and other factors are among the subjects that are discussed as causes of oscillations in biological systems [7–10]. Nevertheless, the question of the mechanism(s) of action of these factors upon biological systems remains a hot topic of scientific discussion.

The objective of this study was the elucidation of rhythms in the entire biological system that would allow one to use it in the future in experiments to investigate the mechanism(s) of the effects of external low-intensity physical factors (including those of a cosmo-physical and geomagnetic nature) on the dynamics of biological processes. Luminous bacteria that exhibit luminescence in the visible range of the spectrum, characterized by high sensitivity and rapid response times to the actions of various factors, which is easy to record instrumentally, are very suitable for this purpose. Pulse-type bioluminescence oscillation rhythms of a single bacterium were described in [11].

Luminous bacteria are a group of microorganisms that are capable of emitting light in the green–blue range of the visible spectrum. This group includes 17 species, with the majority of them being marine species. The luminous bacteria *Photobacterium phosphoreum* are distinct from others in having the most prolonged and intensive emission [12].

Hence, the objective of this study was an investigation of the bioluminescence dynamics of *Photobacterium phosphoreum* under the condition of the growth of the colonies on solid medium at optimal growth conditions.

MATERIALS AND METHODS

The IMV V-7071 strain of the luminous marine bacterium *P. phosphoreum* from the culture collection of the Zabolotnyi Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine was used as an object of the study.

To investigate the bioluminescence dynamics of a single colony of the luminous bacteria growing on a solidified agar-based synthetic medium the following medium composition was used (g/L): peptone, 5.0; yeast extract, 1.0; NaCl, 30.0; Na_2HPO_4, 5.3; $KH_2PO_4 \cdot 2H_2O$, 2.1; $(NH_4)HPO_4$, 0.5; $MgSO_4 \cdot 7H_2O$, 0.1; agar, 20.0; glycerol, 3.0 mL/L; distilled water, up to 1 L, and a pH of 7.6. A detailed protocol for *P. phosphoreum* bacteria cultivation was presented in [13].

The dynamics of the bacterial luminescence intensity were investigated using an experimental setup that was designed in our laboratory. The measurement technique was as follows: the light ($\lambda = 490$ nm from a sample with luminous bacteria was focused on the photocathode of an FEU-115 photomultiplier tube using a fast high-numerical-aperture objective ($A = 0.7$). The voltage applied to the PMT was $U = 1.3$ kV at $I = 1.5$ mA. The PMT anode current was amplified with a precision amplifier that operated in the current–voltage transformation mode. The output voltage from the amplifier was recorded with a multimeter

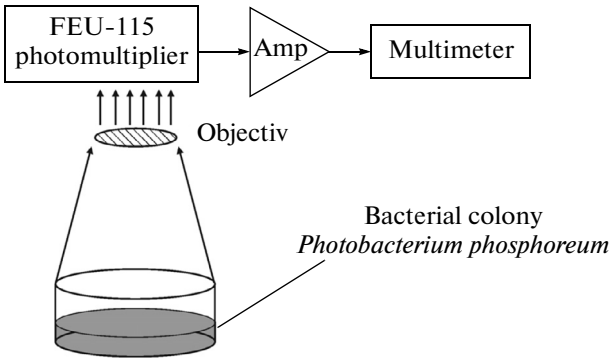

Fig. 1. A schematic representation of the experimental setup. The details are in the text.

with a high input impedance. The schematic representation of the setup for recording bacterial bioluminescence is presented in Fig. 1.

EXPERIMENTAL PROTOCOL

Measurements of the bioluminescence intensity of bacteria were carried out in a dark room. The level of the "dark" signal from the PMT, U_0, was measured initially, the sample with bacteria was then placed under the objective, and the signal of the sample containing a colony of the *Photobacterium phosphoreum* IMV V-7071 bacteria, U_1, was measured. The intensity of the signal from the bacteria exceeded the level of the dark signal by more than two orders of magnitude. The differential signal $U = U_1 - U_0$, which was proportional to the intensity of bacterial luminescence, was used for further analysis.

The bioluminescence intensity was recorded once per minute (with an accuracy of 0.1 s) for 3 hours. The results of analysis were not affected by the frequency of experimental measurements that were used for the analysis. The temperature in the room where the experiments were conducted was maintained at (22.0 ± 0.5)°C. The dynamics of the colony luminescence were investigated at different cultivation times of the cells (at the 3rd, 5th, and 6th days).

The final stage of the investigation was the time-frequency analysis of the intensity of bacterial luminescence.

It must be noted that the results that were obtained during the study were very noisy and were non-stationary in the time scale. All of this created difficulties for the analysis of obtained data using commonly accepted approaches (Fourier transform). In this connection the time-frequency analysis of the experimentally obtained data was conducted using a wavelet transform in the MATLAB 7.11 medium. A Morlet wavelet was used as a basis function of the wavelet transform.

The wavelet transform is a generalization of spectral analysis, of which the classical Fourier transform is a typical representative. The wavelets have significant advantage for the analysis of non-stationary signals over the Fourier transform, which provides us with only global information on the frequencies of the investigated signal. Unlike the traditional Fourier transform, the wavelet transform provides a two-dimensional scan of the investigated one-dimensional signal; the frequency and coordinate (the time of the emergence of this frequency in the experimental signal) are considered as independent variables in the process.

The wavelet transform is used in the cases where the result of the analysis of a given signal should simultaneously contain not only a list of characteristic frequencies, but also information on certain local coordinates (in time) at which these frequencies emerge. Hence, the analysis and processing of non-stationary (in time) or irregular (in space) signals of different types comprises the main area of the application of wavelet analysis.

The comparison of the results that were obtained using the wavelet transform and the classical Fourier transform that we conducted previously showed good agreement between the values of harmonic components that were obtained by different methods [14].

RESULTS AND DISCUSSION

The results of monitoring of the bioluminescence intensity of the IMV V-7071 *Photobacterium phosphoreum* bacteria that were cultivated under optimal growth conditions are presented in Fig. 2a. The wavelet analysis of the experimental data showed (Fig. 2b) that the dynamics of the bioluminescence intensity was governed by certain regularities and demonstrated a quasi-periodic character (Fig. 3). The results that were obtained in this work indicate that certain periods were observed in the intensity of bioluminescence. The values of the periods of the bacterial luminescence intensity that were found in this work are: 6–9, 11–13, 15–18, 21–29, 30–39, 40–55, and ~60 min. The amplitudes of these oscillations vary up to 10% of the average level of the bioluminescence (Fig. 3).

To assess the effect of the measurement instrumentation on the observed quasi-harmonic character of the bacterial luminescence, we conducted additional experiments. The dynamics of the emission intensity of the stable light source (the emission level corresponded to the level of bacterial luminescence) was compared with the dynamics of bacterial bioluminescence. As well, the dynamics of the dark-signal intensity of the photomultiplier was compared with the dynamics of the bacterial luminescence. The comparison of the results of wavelet analysis of all the mentioned dynamics indicates that the found periods are observed only in the dynamics of the bacterial bioluminescence.

It must be noted that the period values that were detected during cultivation of the *P. phosphoreum*

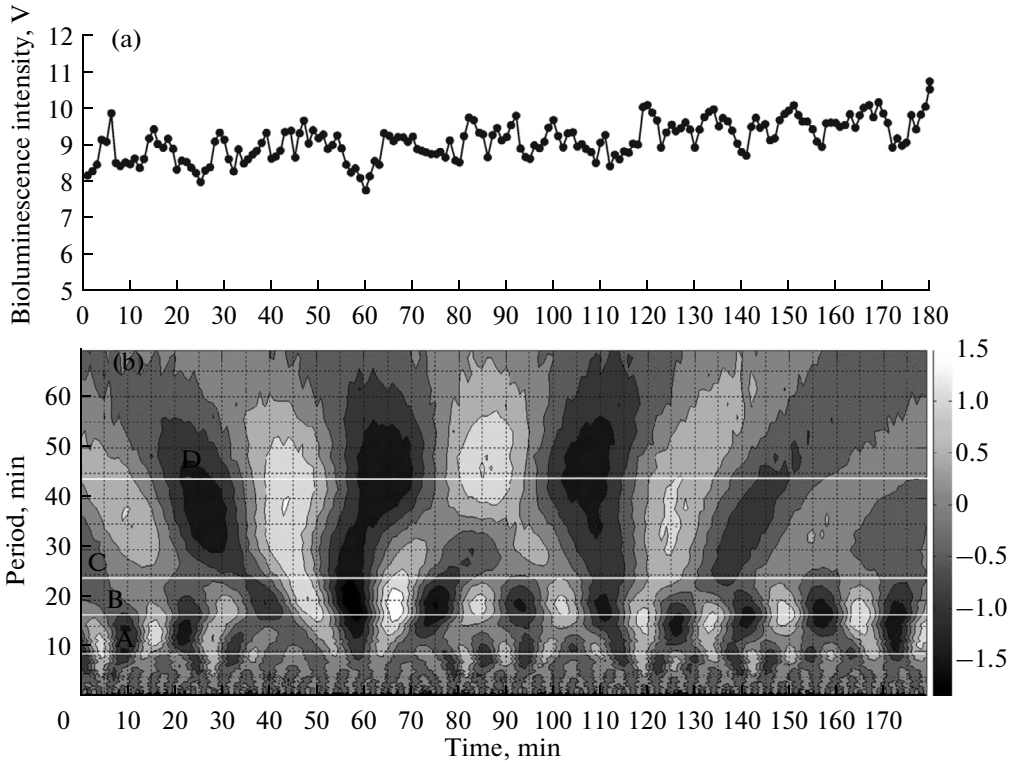

Fig. 2. The dynamics of the bioluminescence intensity of the IMV V-7071 *Photobacterium phosphoreum* bacteria. (a), the actual dynamics of the *P. phosphoreum* bacteria luminescence intensity, (b), the wavelet transform of the dynamics.

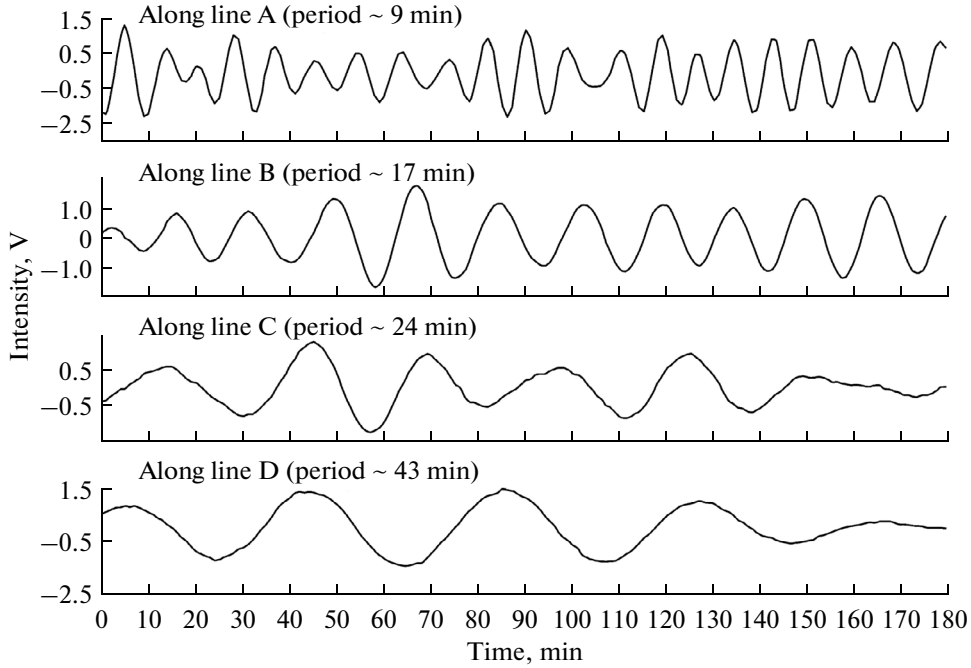

Fig. 3. The multimodal character of the dynamics of the bioluminescence intensity of *Photobacterium phosphoreum* IMV V-7071 bacteria (harmonic components of the dynamics of the luminescence intensity along the respective lines in Fig. 2b). The amplitudes of the harmonics are calculated from the average level of the bioluminescence intensity in absolute units.

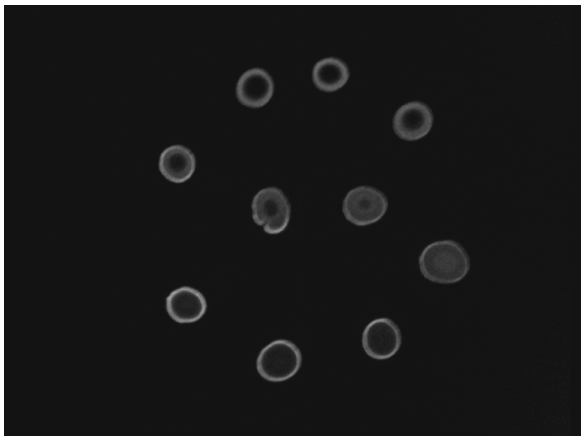

Fig. 4. A photograph of differentiated bacterial colonies of the IMV V-7071 *Photobacterium phosphoreum* bacteria.

IMV V-7071 colonies demonstrated good repeatability, but were not present as an entire set in all of the experiments. To explain this fact, an understanding of the mechanism of the observed rhythms is required, however, this was not within the scope of this study.

Analysis of the luminescence dynamics at different times of bacteria cultivation showed that the harmonic component with the period of ~60 min disappeared from the luminescence dynamics with the increased age of the culture and a decrease of the amplitude of the revealed periods was observed. This can be the result of a decline of the intensity of metabolic processes in an aging bacterial culture. Differentiation of the cells in the growing colony was reflected in the intensity of their luminescence. The cells in the peripheral growth zone that were younger and had an active metabolism demonstrated bright luminescence. At the same time, the central part of the colony contained less viable cells (Fig. 4). The experiments that were conducted earlier with this culture under conditions of a liquid nutrient medium showed that the maximum luminescence of the cells was observed during the exponential growth phase [15]. These data confirm the well-known hypothesis that bacterial luminescence is an integral indicator of the activity of metabolic processes that occur in the cell [16]. Despite the spatial–temporal heterogeneity of the cells in the colony of the *P. phosphoreum* IMV V-7071 during the luminescence experiments the total level of luminescence in the considered time period was unchanged due to the growth of new biomass.

The periodicity in the luminescence changes suggests the existence of rhythms in the activity of the main enzyme of the luminescent reaction, viz., luciferase. Hourly rhythms of protein synthesis [17] that reflect the ratio of sol and gel in the cell [18] similar to the faster intracellular rhythms could be one of causes of this phenomenon. According to S.L. Zaguskin [18] the periodicity of structural rearrangements in water and aqueous solutions is one of the important conditions of sol–gel phase transitions.

Hence, the changes of the amplitude of the spectral components in the bioluminescence dynamics could be related with the heterogeneity of bacterial cells, which emerges during the later stages of colony development; nevertheless, this phenomenon requires a separate investigation.

IN CONCLUSION

The periods that were found in this work in the dynamics of the *P. phosphoreum* IMV V-7071 bacteria bioluminescence are in good agreement with the values of the biological periods of the entire organism [3–5], as well as with the periods of the changes of the physicochemical properties of water that were found in [19] (1–3, 5–9, 10–13, 14–19, 21–29, 30–39, 40–55, and ~60 min). This agreement of the periods of biological activity of the entire organism [3–5] with the bioluminescence of the *P. phosphoreum* IMV V-7071 bacteria, and the variability of the physicochemical properties of water [19] allows us to suggest that these processes are related.

From the evolutionary point of view, these are exactly the cosmo-physical rhythms that could serve as the leading synchronizing factors of biological processes. Periods of solar pulsation on the minute scale are known: ~12, ~17, ~27, ~32, ~45, and ~57 min [7, 20]. The periods of the bioluminescence intensity that we found, the periods of biological activity of the entire organism that were obtained earlier [3–5], and the periods of changes in the water physicochemical properties [19], as well as the good agreement of these values with the rhythms of solar weather [7, 20] is indicative in our opinion of the important role of water in the transmission of signals of a physical nature to biological objects. A correlation between geomagnetic storms and the bioluminescence activity of bacteria was shown earlier in [21, 22].

Summing up all the above, it can be suggested that cosmo-physical factors (affecting mainly the Earth's magnetic field and its variations) synchronize the functions of the cells of any biological organism. The effect of a magnetic field that penetrates directly to the level of each cell can be directed to any of the magnetic moments that are present in a biological system (the electron spin of a free radical, nuclear magnetic moment, orbital magnetic moment, magnetic moment of *ortho* water molecules, etc.). These magnetic moments become in a sense "antennas" of biological objects that can perceive the governing effects of external factors [23].

CONCLUSIONS

1. Analysis of the obtained data indicates the existence of rhythms in biological processes that occur in the

growing culture of the IMV V-7071 *P. phosphoreum*. The observed rhythmicity demonstrates a multimodal character and is in agreement with the periods of the changes of physicochemical characteristics of water.

2. The revealed quasi-periodic character of the bacterial bioluminescence allows us to propose IMV V-7071 *P. phosphoreum* as a test biological system for further investigation of the effects of low-intensity external factors (including those of cosmophysical and geomagnetic origins) on biological processes.

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