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BACTERIAL LYSATE OF *Lactobacillus rhamnosus* DV SUPPRESSES BIOLUMINESCENCE OF *Photobacterium phosphoreum*

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Background: Recent studies on the lysate of *Lactobacillus rhamnosus* DV have demonstrated its antimicrobial and fungicidal activities. Classical microbiological methods for evaluating postbiotics and metabiotics are resource-intensive that need for new rapid and sensitive tests systems to assess their antimicrobial and antifungal properties.

Objectives: The aim of this work was to study the effects of the *Lactobacillus rhamnosus* DV lysate on the intensity of *Photobacterium phosphoreum* bioluminescence and to demonstrate the potential use of bacterial bioluminescence to determine the bacteriostatic activity of this postbiotic substance.

Materials and methods: The cell lysates of lactic acid bacterial strain *L. rhamnosus* DV were investigated, which were included in the Del-Immune V® dietary supplement produced by MirImmunoPharm LLC (Ukraine) in cooperation with Stellar Biotics, LLC (USA). The culture of *P. phosphoreum* IMV B-7071 was used in this study. Bioluminescence measurements were conducted using a digital photo-registration method. Bioluminescence was analyzed using cross-platform open-source software ImageJ (NIH) in brightness units in RGB color space.

Results: The bioluminescence intensity initially increased with population density during the first day, then faded despite the continued growth of cell density in the bacterial suspensions. The addition of *Lactobacillus rhamnosus* DV lysate significantly suppressed bioluminescence, reducing it to background levels within 24 hours, with the effect varying by concentration. The peak luminescence in control samples occurred at 18 hours, whereas in lysate-treated samples it shifted to 3–6 hours, depending on the lysate concentration.

Conclusions: The lysate of *Lactobacillus rhamnosus* DV causes a significant decrease in the growth rate of *Photobacterium phosphoreum* and strongly suppresses the intensity of their bioluminescence, the magnitude of which depends on the concentration of the lysate. This finding supports the use of bacterial luminescence as an effective way for evaluating the bacteriostatic activity of postbiotic substances against pathogenic and opportunistic microorganisms.

KEY WORDS: postbiotics; bacterial lysate; bioluminescence; *Lactobacillus rhamnosus*; *Photobacterium phosphoreum*.

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Currently, there is a large array of data on the beneficial properties of probiotics in the regulation of metabolism [1]. At the same time, postbiotics and metabiotics are substances containing inactivated microorganisms or their components, which are safe for use and have a positive impact on human health [2, 3].

The term 'postbiotic' is used to refer to a cell-free supernatant with bacterial final or intermediate products of metabolism produced by living bacteria or formed in the process of lysis of bacterial cells. The term 'metabiotics' is often used to denote fragments of the cell wall of probiotic microorganisms and DNA fragments remaining after the destruction of probiotic cells [4,5]. Due to their properties, postbiotics and metabiotics provide a local effect on the tissues of the intestinal epithelium and affect various organs and systems of the body. It is important for postbiotic metabolites and structural cell's fragments of metabiotic cells mimic to display the therapeutic effect of probiotics, which allows avoiding the risks associated with the introduction of live microorganisms into an organism especially with weakened immune defenses.

Postbiotic supplements can be beneficial for irritable bowel syndrome, inflammatory bowel and respiratory diseases [6]. Postbiotics and metabiotic substances, obtained by different technology from probiotics during probiotic's manufacturing process also demonstrate antibacterial, anti-inflammatory, immunomodulatory, antiproliferative, and antioxidant activity [6, 7]. Such postbiotics, like probiotic lysates, have significant advantages as safe substances with high immunotherapeutic efficiency. In particular, they activate certain aspects of cellular and humoral immunity [8].

Recent studies on the lysate of *Lactobacillus rhamnosus DV* have demonstrated its antimicrobial and fungicidal activities [9]. This activity is attributed to antibacterial protein compounds known as bacteriocins, which are produced by bacteria to combat other competitive bacterial species. Bacteriocins play a crucial role in interspecific interactions and competition among bacteria and hold significant potential in medicine and biotechnology [10]. However, the use of classical microbiological methods to evaluate the bacteriostatic and antibacterial properties of postbiotics is costly and requires specific resources and working time. Therefore, the development of sensitive and rapid test systems for assessing the antimicrobial and antifungal activities of postbiotics is a pressing need.

Photobacterium phosphoreum is a well-established model in ecotoxicology and biomedical research, owing to its high sensitivity to diverse chemical and physical environmental stimuli [11]. *P. phosphoreum* generates light through a flavin oxidation reaction catalyzed by bacterial luciferase. In the presence of some toxic for human substances, the intensity of bioluminescence changes. But first of all, the bioluminescent activity of *P. phosphoreum* serves as a sensitive indicator of the presence of toxic substances specifically to this type of bacteria.

On the other hand, one can expect a corresponding reaction of *P. phosphoreum* to the influence of postbiotic preparations that contain bacteriocins and other biologically active compounds and demonstrate bacteriostatic and antibacterial activity. In this regard, the aim of this work was to study the effects of the *Lactobacillus rhamnosus DV* lysate on the intensity of *P. phosphoreum* bioluminescence to explore to demonstrate the potential use of bacterial bioluminescence to determine the bacteriostatic or/and antibacterial activity of postbiotic preparations.

MATERIALS AND METHODS

In this research the cell lysates of lactic acid bacterial strain *L. rhamnosus DV* were investigated, which were included in the Del-Immune V® dietary supplement produced by MirImmunoPharm LLC (Ukraine) in cooperation with Stellar Biotics, LLC (USA). Content of *L. rhamnosus DV* lysate lyophilized powder: protein 100 mg/g; DNA 118 mg/g; muramyl

peptides 15.0 – 18.0 mg/g; pH of 1% suspension 6.5–7.5; moisture (%) no more 5.0. The dietary supplement that contains the cell lysate of lactic acid bacterial strain *L. rhamnosus DV* is packed in capsules and contains insoluble components. Its solutions at high concentrations form turbid colloidal dispersions.

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 21–23°C in the liquid nutrient medium of the following composition (g/L): peptone (LLC “Pharmaktiv”, Ukraine) — 5.02, yeast extract (LLC “Pharmaktiv”, Ukraine) — 1.0; NaCl — 30.0; Na₂HPO₄ — 5.3; KH₂PO₄ x 2H₂O — 2.1; (NH₄)₂HPO₄ — 0.5; MgSO₄ x H₂O — 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 photocolorimeter 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 10^7 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 10^7 cells/ml.

Aqueous solutions of *L. rhamnosus DV* lysate (lyophilized powder) were added to the experimental bacterial samples during the lag phase to reach final concentrations of 0.1, 0.3, 1.0, 3.0, 10.0, and 30.0 mg/ml. Corresponding volumes of water were added to the control samples. The final volume of each bacterial suspension in the plastic wells was 3 ml.

Bioluminescence measurements were conducted using a digital photo-registration method with a Canon 700D camera, set at ISO 12000, an aperture of 5.6, and an exposure time of 2 seconds. Background (baseline) bioluminescence was measured without stirring the bacterial suspension at 3, 6, 18, 24, and 30 hours after the experiment began. Bioluminescence parameters were analyzed using ImageJ (NIH), a cross-platform, open-source software designed for image processing and analysis in biological, medical, and other research fields. Bioluminescence intensity was measured in brightness units in RGB color space. In 8-bit imaging, the intensity of each RGB channel and the resulting grayscale average are represented by integer values ranging from 0 to 255. We used the mean value of bioluminescence intensity averaged over 3 channels and also used brightness values for individual color channels.

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 by two sample Kolmogorov-Smirnov test which compares not only means, medians, or ranks, but also takes into account differences in the shape, skewness, or variance of two distributions. The statistical sample size for each type of sample was $n = 8$.

RESULTS AND DISCUSSION

The results of the study on the time dynamics of *P. phosphoreum* bacterial suspensions indicate the phase evolution of their bioluminescence. During the first day, the intensity of bioluminescence increased in parallel with the growth of the bacterial population density. After this period, the bioluminescence gradually faded, despite the continued increase in the population density (Figs. 1 and 3). This is consistent with previously obtained data on the temporal dynamics of *P. phosphoreum* bioluminescence over an extended period [12].

The primary intensity of the light signal falls within the blue and green ranges, where the principal spectral band of bacterial luminescence is located [12,14]. The contribution of the red range typically remains at a background level, not exceeding 10–15 conventional RGB units. Consequently, changes in the average bioluminescent signal intensity are mainly due to the activity of bacterial luciferase. The addition of *L. rhamnosus* DV lysate significantly suppressed the average intensity of bacterial luminescence over 24 hours, and after a day, the bioluminescence was barely observed and subsequently faded to background levels (Fig. 1).

The effect of the lysate on *P. phosphoreum* bioluminescence depended on its concentration. The greatest effect of the lysate was observed during the peak luminescence phase of the photobacteria, which occurred after 18 hours of incubation in the control samples. Additionally, the lysate not only significantly reduced luminescence intensity but also altered its temporal dynamics. While in the control samples, the peak luminescence occurred at 18 hours, in samples containing the lysate, the peak luminescence phase occurred at 3–6 hours, depending on the concentration of the lysate (Fig. 1). At a lysate concentration of 0.1 mg/ml, maximum luminescence was observed at 6 hours, whereas at higher concentrations, it was observed at 3 hours. At the highest concentrations of 10 and 30 mg/ml, the luminescence phases were almost not observed due to nearly complete suppression of bacterial luminescence.

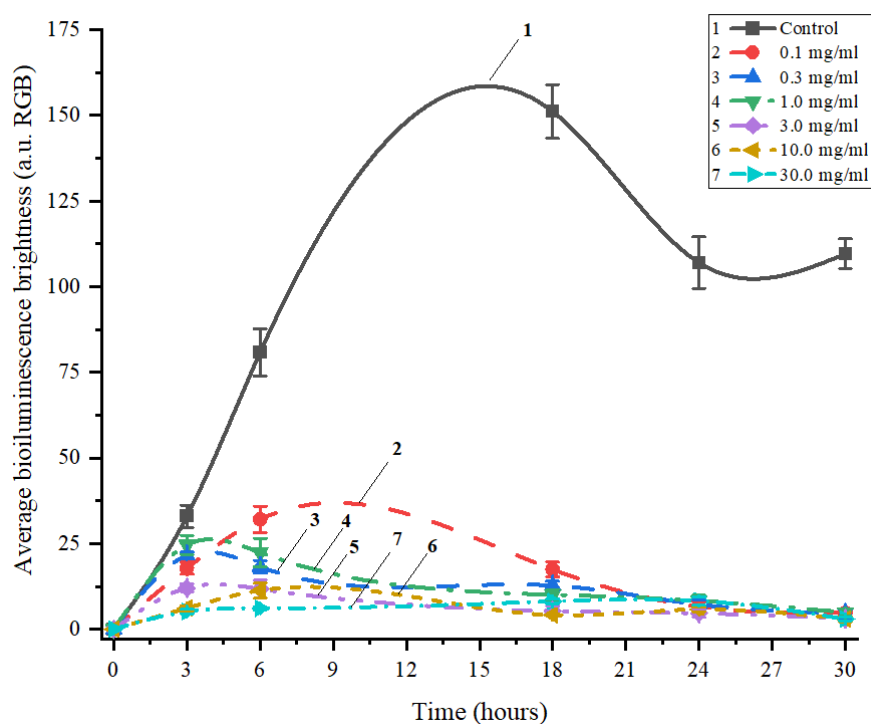


Fig. 1. Temporal dynamics of the average intensity of bioluminescence of *P. phosphoreum* bacterial suspensions in control groups and in the presence of *L. rhamnosus* DV bacterial lysate at different concentrations. *Notes:* Solid and dashed lines represent spline approximation of the times dynamics.

The concentration dependence of the lysate's effect on the bioluminescence intensity of *P. phosphoreum* is clearly illustrated in Fig. 2. According to the data presented in the figure, the lysate significantly suppresses the bioluminescence intensity of photobacteria by 4–5 times even at low concentrations. The concentration dependence of the lysate's effect on photobacteria luminescence has an exponential character and is well linearized when the concentration axis is logarithmically scaled (Fig. 2). This dependence is described by the equation

$I = 14.63 - 2.89 \ln[C]$. The model demonstrates the high biological activity of the lysate in

suppressing bioluminescence, which, according to theoretical calculations, can be detected at concentrations as low as 10^{-5} – 10^{-4} mg/ml.

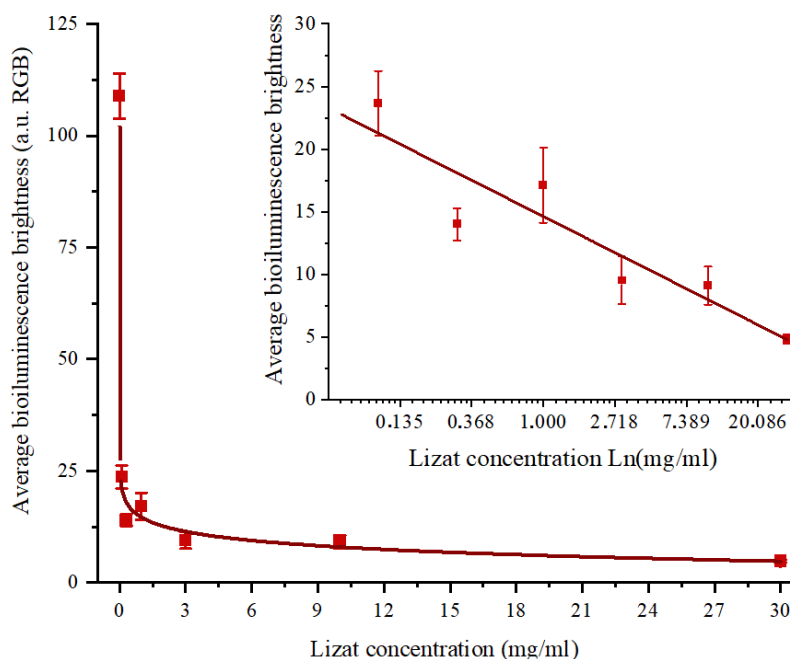


Fig. 2. Dependence of the average bioluminescence intensity of *P. phosphoreum* on the concentration of *L. rhamnosus* DV lysate during the peak luminescence phase of the control samples.

Notes: The solid line represents the logarithmic approximation of the concentration dependence. The inset graph shows the result of the logarithmic linearization of the concentration dependence.

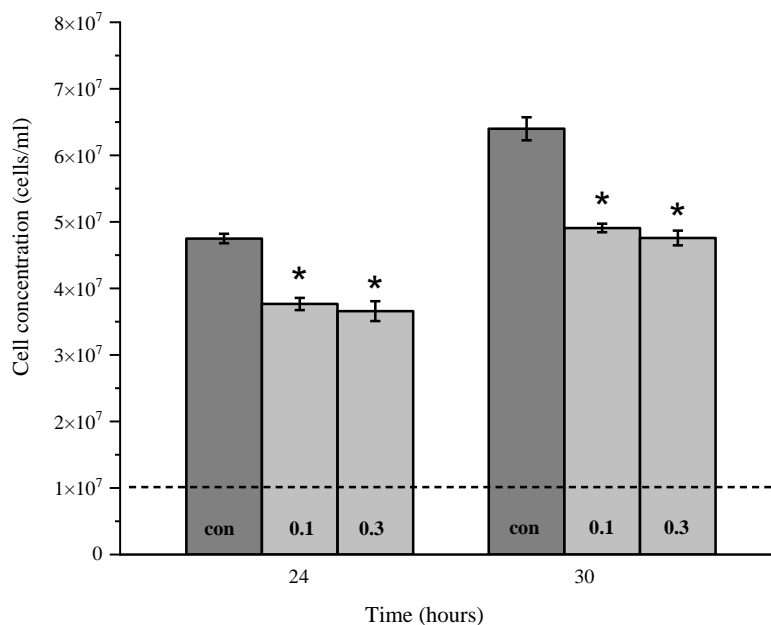


Fig. 3. Effect of *L. rhamnosus* DV bacterial lysate at concentrations of 0.1 and 0.3 mg/ml on the density of *P. phosphoreum* bacterial population after 24 and 30 hours of development.

Notes: con – контроль; * – $P < 0.01$ compared to control samples. The dashed line indicates the initial cell concentration level in bacterial suspensions for all samples. In samples with higher concentrations of lysate, it is not possible to measure cell concentration using optical methods based on light scattering due to the high turbidity of the lysate at these concentrations.

The high inhibitory activity of the lysate on the bioluminescence of *P. phosphoreum* raises questions regarding the underlying mechanism of this phenomenon and its impact on cell density. Is the reduction in bacterial bioluminescence intensity a result of inhibited population growth, or does the lysate interfere with metabolic pathways associated with the bacterial luciferase system?

Figure 3 presents the results of the study on the effect of *L. rhamnosus* DV lysate at concentrations of 0.1 and 0.3 mg/ml on the density of the *P. phosphoreum* bacterial population after 24 and 30 hours of development. The lysate at these low concentrations reduced bacterial density by approximately 25%, indicating a bacteriostatic effect through the suppression of not only metabolism but also bacterial reproduction. Nevertheless, the lysate suppressed bioluminescence intensity by 75–80%, suggesting that the bacterial luciferase system is highly susceptible to the lysate's inhibitory action, beyond its general bacteriostatic effect.

This sensitivity is likely related to the specific structural and functional organization of the bioluminescent system in photobacteria. Bacterial bioluminescence is mediated by a reaction catalyzed by luciferase (a flavin-dependent monooxygenase), which requires reduced flavin mononucleotide (FMNH₂), O₂, and a long-chain fatty aldehyde (RCHO). The reaction products are oxidized flavin mononucleotide (FMN), the corresponding fatty acid (RCOOH), H₂O, and visible light [13]. This process is controlled by the lux A, B, C, D, E operons. It was found that the structural genes lux A, B encode luciferase, and lux C, D, E encode aldehyde synthesis. This part of the complex is the same for all studied bacteria [14]. The role of luminescence in microbial physiology remains poorly understood. However, its involvement in aquatic organism protection, intercellular communication, and stress response mechanisms has been widely discussed in the literature [15, 16]. Thus, the bioluminescence of photobacteria is a highly energy-consuming process that sensitive to any changes in the energy supply to the cells. This process is inhibited under adverse conditions caused by various environmental stressors or toxic agents affecting this species. Based on this fact and literature data array, we assume the main factor causing the suppression of bioluminescence and reproduction of *P. phosphoreum* are bacteriocins together with other biologically active components contained in the lysate.

CONCLUSIONS


L. rhamnosus DV lysate causes a moderate decrease in the growth rate of *P. phosphoreum* while strongly suppressing its bioluminescence intensity in a dose-dependent manner. The effectiveness of the lysate follows an exponential pattern, suggesting threshold effects at concentrations as low as 10⁻⁶–10⁻⁴ mg/ml. These findings substantiate the feasibility of using bioluminescence assays to evaluate the antimicrobial potential of postbiotic preparations. Furthermore, they provide a foundation for novel applications of bioluminescent bacteria in medicine and biotechnology, specifically for developing standardized methods to assess the activity of biotechnological products against pathogenic and opportunistic microorganisms.


CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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ВПЛИВ БАКТЕРІАЛЬНОГО ЛІЗАТУ *Lactobacillus rhamnosus* DV НА БІОЛЮМІНЕСЦЕНЦІЮ *Photobacterium phosphoreum*

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Актуальність: Нещодавні дослідження лізату *Lactobacillus rhamnosus* DV продемонстрували його антимікробну та фунгіцидну активність. Класичні мікробіологічні методи оцінки постбіотиків та метабіотиків є ресурсоємними та потребують нових швидких та чутливих тест-систем для оцінки їхніх антимікробних та протигрибкових властивостей.

Мета: Метою цієї роботи було вивчити вплив лізату *Lactobacillus rhamnosus* DV на інтенсивність біоломінесценції *Photobacterium phosphoreum* і продемонструвати потенційну можливість використання бактеріальної біоломінесценції для визначення бактеріостатичної активності постбіотичних препаратів.

Матеріали та методи: Досліджено клітинні лізати штаму молочнокислих бактерій *L. rhamnosus* DV, що входять до складу харчової добавки Del-Immune V® виробництва ТОВ «МірІмуноФарм» (Україна) у співпраці зі Stellar Biotics, LLC (США). У цьому дослідженні використано культуру *P. phosphoreum* IMV B-7071. Вимірювання біоломінесценції проводили методом цифрової фотореєстрації. Біоломінесценцію аналізували за допомогою кросплатформного програмного забезпечення з відкритим кодом ImageJ (NIH) в одиницях яскравості в колірному просторі RGB.

Результати: Інтенсивність біоломінесценції спочатку зростала зі збільшенням щільності популяції протягом першого дня, а потім зменшувалась, незважаючи на подальше зростання щільності клітин у бактеріальних суспензіях. Додавання лізату значно пригнічувало біоломінесценцію, знижуючи її до фонового рівня упродовж 24 годин, причому ефект змінювався залежно від концентрації. Пік люмінесценції в контрольних зразках спостерігався через 18 годин, тоді як у зразках, оброблених лізатом, він зміщувався до 3–6 годин залежно від концентрації лізату.

Висновки: Лізат *Lactobacillus rhamnosus* DV викликає значне зниження швидкості росту *Photobacterium phosphoreum* та сильно пригнічує інтенсивність їхньої біоломінесценції, величина якої залежить від концентрації лізату. Цей факт підтверджує ідею використання бактеріальної люмінесценції як ефективного способу оцінки бактеріостатичної активності постбіотичних речовин проти патогенних та умовно-патогенних мікроорганізмів.

КЛЮЧОВІ СЛОВА: постбіотики; бактеріальний лізат; біоломінесценція; *Lactobacillus rhamnosus*; *Photobacterium phosphoreum*.