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The Poly(vinyl alcohol)-Immobilized Photobacteria for Toxicology Monitoring

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ABSTRACT

The present work is undertaken to studies the important factors affecting the stability of a light emission at immobilized photobacteria, and application PVAGs luminous biosensors for biomonitoring of toxicants. The intensity and stability of a light emission is competently controlled by: 1) intensity and persistens of a luminescent cycle using bacterial strain; 2) type of the carrier and the composition of the gel-formation medium; 3) freeze-thawing procedures; 4) physical and chemical conditions of storage and application.

Keywords: Component; Bioluminescence; Photobacteria; Immobilization; Poly (Vinyl Alcohol)

1. Introduction

The luminous bacteria are widely used as a biosensor for biomonitoring of toxicants [1-3]. The application of photobacteria is defined by high sensitivity of bioluminescence to a wide spectrum of toxic substances, and a fast responsibility of light reaction. Bioluminescent activity reflects of a cell metabolism to chemical compounds with toxic action, including the heavy metals, aliphatic and aromatic hydrocarbons, phenols and a number of other xenobiotics. The emission value serves as the quantitative indicator of the general and specific toxicity of the sample.

Light-emitting bacteria can be used for toxicity monitoring both using the free and immobilized forms. The immobilization of photobacteria allows to raise stability of light emission by biosensors, what is could form the basis of a cost-effective, real-time system for detecting environmental pollutions [4,5]. Many diverse gel matrices has been proposed as possible carriers for immobilisation of luminous bacteria, In this case, either natural: agar, agarose, alginate, carrageenan, gelatin, collagen, or synthetic (polyacrilates, polyurethanes, polyethers) bio- polimers has been used as the gel-forming agents [6,7].

Taking into account that a luminescence activity extremely sensitive to temperature influence, in quality gel-forming agent is chosen poly (vinyl alcohol) (PVA). The crioPVA gel formation proceeds at negative temperatures. This carrier on structural, physical and chemical properties possess a number of advantages in comparison with other carriers. PVA gels have very high micro-and macro-porosities which provide favored conditions for the non hindered mass transfer of substrates and toxicants. Thermo-stability of cryoPVA gels exceeds that of other commonly employed thermo-reversible gel carriers, such as agar, agarose, ets. PVAGs are highly resistant to biological degradation and practically insensitive to media composition, — solutes, buffers, pH. PVA itself is a biologically compatible, nontoxic, readily available low-cost polymer. Rheological parameters of cryoPVAGs are quite stable and allow for this carrier to by used under variety biomonitoring conditions [6,7].

The aim of this study was the analyses of the factors affecting the stability of a light emission at PVA-immobilized photobacteria and application PVAGs luminous biosensors for biomonitoring of toxicants.

2. Materials and Methods

Bacterial strains, media and culture grow conditions. The strains used in this study were *Photobacterium phosphoreum* strain №331 MC MSU

Immobilization procedures. The immobilization was carried out according to a technique [6, 9]. Liquid biomass were mixed with 13% solution of poly(vinyl alcohol), (Mm – 48000 Da), Gel solution were prepared at 24h in 1) the GM media; 2) Na-phosphat buffer + 2% NaCl, pH 7.6, and 3) 3% NaCl. The 0.2 ml gel-cell solution pour out on 96-hole planshet, and transferred to the freezing chamber (20°C) for gel formation with simultaneous inclusion of cells in a polymeric matrix. The final cell concentration in granules about $1–5\times10^7$ cells/granule. Prior to each measurements samples were defrosted at 4°C over 24 h and placed on incubation media, and stored at 4°C. The procedures of cryogenic gel-formation and cell reactivation in details are described in [9].

Measurement of light intensity and concentration of cells. A bioluminescence registered on luminometer 1250 LKB-Wallac and expressed in relative units, or absolute values of photons output according the standard [10]. The cell contain carried out under maintenance ATP using a bioluminescent method with firefly luciferase [11]

3. Results

CryoPVAGs-cell immobilization.

The intensity and duration of light output by the isolated cells is defined not only by natural emission properties of strains and composition of incubation mixture. Except energet-

ically substrates, the luminescence is complexly controls by the ionic force, temperature and pH.

It is obvious, that the composition of gel-formation media should influence on cells stability in the carrier and metabolic activity first of all on stages of gel formation. For a choice of the optimal media composition and conditions, three difference mixtures (GM-medium with and without pepton, and 3% NaCl) has been used for gel formation, and the kinetics of light emission after "freezing/thawing" procedure was carried out. Criteria were: initial intensity of a luminescence of granules, the general integrated excitation of photons in the course of storage of preparations to residual level of a luminescence less than 0.1% from initial, and the rate of decay.

The developed technology cryogenic gel formation has allowed to keep survival of luminous bacteria in the carrier, practically on 100%, without introduction additional crioprotecting agents and procedures of a light induction. Specific bioluminescent activity was restored to level of activity of free cells (~10^5 photons/sec. per cell). At storage at –80°C bioluminescent activity remained without changes in a current of 2 years. The detected level of light emission of psychrophilic strains at 4°C — over 1 month, at 20°C — 3 days. The analysis of the specific activity of cells after a freezing/thawing procedure and stabilization of light emission at 4°C (24 hours from the beginning the heating stage), have shown, that most effective gel formation mixture is the culture growing media. In this case the immobilized bacteria kept practically 100% level of luminescent activity. The exception of peptone causes decrease in specific luminescent activity on 1–1.5 order. The most essential recession of a luminescence (in ~10^3 times) was observed when the gels formation process has come with only 3% NaCl.

Thus the composition of the gel formation media, not incubation mixture, makes the basic impact on intensity and duration of bioluminescence of immobilized preparations. Concentration of the carrier (5, 7, 10%) does not change emission and kinetics parameters. The analysis of stability of PVA-immobilized cells of photobacteria at storage at low temperatures spent with use of the preparations formed in growth culture media. It is established, that at –80°C remained practically 100% level of bioluminescent activity of granules (2 years of supervision), at –20°C activity falling on 20% in a current of year was observed, however residual activity and in this case was on much higher (more, than on two order) level, than residual activity of the preparations stored at 4°C. As essential distinctions in inhibition kinetics is not revealed, the time parameters postulated to free cells are chosen for the toxicity analysis with granules.

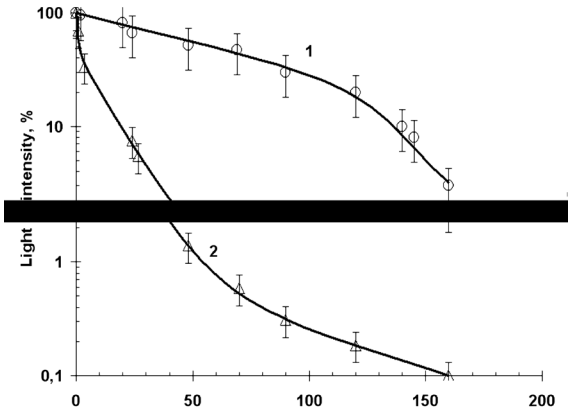

Figure 1. The time dependence of a specific bioluminescent activity during incubation at 4°C in 3% NaCl immobilized (1) and free (2) cells of photobacteria.

The optimized conditions of reception and storage immobilized cells formed a basis of use of luminescent granules as biosensor controls. The analysis of inhibition kinetics from free and immobilazed cells has shown similar time profiles, testifying to absence serious diffusion restrictions of a gel material and the form of granules for all types of molecules. Supervision logically follows from structural characteristics of the matrix having macropores. As essential distinctions in inhibition kinetics is not revealed, the time parameters postulated to free cells are chosen for the toxicity analysis with granules. The table illustrates the immobilized cells bioluminescence inhibition by various classes of toxins.

4. Discussion

For photobacteria, at which temperature optimum of a luminescence 15–25°C (depending on a strain), temperature influence is critical. Most often used in immobilization photobacteria agar, agarose and alginate gels are formed in a range of positive temperatures that is capable to have negative influence on luminescence activity of cells directly during immobilization procedure. Essential value for viability of sea photobacteria plays salt structure of environment. Optimum concentration for light activity of a cells of 2–6% of chloride sodium. The stability of Ca- and Sr-alginate gels at storage and application is also influenced by partial replacement of Ca^{+2} and Sr^{+2} by Na^{+} ions [8]. Glycerol has been used as a necessary component for immobilisation process and to more effective storage.

PVA-cryogels on structural properties [6,7] possess a number of the characteristics having basic value for light emission of photobacteria.

Toxicant	Bioluminescence response, detection range, mg/l	
	5 min	15 min
Phenol	100–600	100–400
Cu^{2+}	5–40	1–8
Zn^{2+}	10–60	0.5–4
Hg^{2+}	0.1–0.6	0.05–0.10
Pentachlorophenol	0.2–2.0	0.05–0.4
2,4-Dichlorophen oxyacetic Acid	1.0–10.0	0.5–10.0
2,4,5-Thrichlorophenoxyacetic Acid	0.5–4.0	1.0–8.0

The basic advantage consists that gel formation process

proceeds at negative temperatures. The PVA-gels possess high thermo stability in a wide range of positive temperatures up to 80°C, that creates possibility of use of a bioreactor in different temperature modes. Physical and chemical parameters of carrier slightly depend on structure of the environment of formation of gel, in particular salt, which has great value for bioluminescent activity of bacteria. The PVA-gels has note been damage by microorganisms. Essentially, that PVA it is nontoxic in relation to the included photobacteria. Consequently, these gel materials can be considered as very promising carrier for use in photobacteria entrapment technologies

The results presented testify the long-term stability and high intensity of a luminescence with PVA-Gs immobilized psychrophilic strains of photobacteria. It is established, that the major importance for stability of a luminescence has a choice of photobacteria strain and of the gel formation mixture. A composition of incubation medium, pH, and especially temperature, have complex an effect as on intensity and duration of a luminescence. Results suggests that the most stability of light output by immobilized preparations can be achieved with incubation at relative low (no more then 20°C) temperature in alkaline (pH 8.5) mixtures.

High survival of cells, with preservation of specific activity of light emission, at level of the free cells, presented to the given work, reflect advantages cryogenic immobilization photobacteria in PVA carrier and application PVAGs-lum biosensors for biodetection of toxicants

REFERENCES

[1] Bulich AA (1979) Use of luminescent bacteria for determining toxicity in aquatic environments. In: Markings LL, Kimerle RA (eds.) Aquatic Toxicology. American Society for Testing and Materials, Philadelphia

[2] Lee JH, Mitchell RJ, Kim BC, Cullen DC, Gu MB (2005) A cell array biosensor for environmental toxicity analysis. Biosens Bioelectron 21(3):500–507

[3] Scheller F, Schubert F (1992) Biosensors. Techniques and instrumentation in analytical chemistry. Elsevier, Amsterdam 11:359

[4] Park KS, Baumstark-Khan Ch, Rettberg P, Horneck G, Rabbow E, Gu MB (2005) Immobilisation as a technical possibility for long-term storage of bacterial biosensors. Radiat Environ Biophys 44:69–71

[5] Lee B, Lee J, Shin D, Kim E (2006) Statistical optimization of bioluminescence Photobacterium phosphoreum KCTC 2852. Environ Int *32(2):265–268*

[6] Lozinsky VI, Zubov AL, Titova EF (1996) Swelling behaviour of poly(vinyl alcohol) cryogels employed as matrices for cell immobilization. Enzyme Microb Technol 18(6):561-569

[7] Lozinsky VI, Plieva FM (1998) PoIy(vinyl alcohol) cryogels employed as matrices for cell immobilization. 3. Overview of recent research and developments. Enzyme Microb Technol 23:227–242

[8] Chun UH, Simonov N, Chen Y, Britzb ML (1996) Continuous pollution monitoring using Photobacterium phosphoreum. Resour Conserv Recycl 18:25–40

[9] Efremenko EN, Senko OV, Kuts VV, Alenina KA, Kholstov AV, Ismailov AD (2010) The luminescent biocatalyst for detecting toxicants. Patent RU 2394910

[10] Hastings JW, Weber G (1963) Total quantum flux of isotropic sources. Opt Soc Am 53(12):1410–1415

[11] Efremenko EN, Tatarinova NYu (2007) The effect of long-term preservation of bacterial cells immobilized in poly(vinyl alcohol) cryogel on their viability and biosynthesis of target metabolites. Microbiol 76(3):383–389

