IMMUNOLOGY AND MICROBIOLOGY

Effect of Human Serum on Bioluminescence of Natural and Recombinant Luminescent Bacteria

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 138, No. 9, pp. 311-315, September, 2004
Original article submitted December 23, 2003

Biphasic modification of bacterial bioluminescence by human serum was revealed: bioluminescence was inhibited at high concentrations of the serum and stimulated at low concentrations. Effects of temperature and duration of exposure on bioluminescence manifested in stimulation of the inhibitory effect at higher temperature and longer exposure. The degree of inhibition of bioluminescence under in the presence of serum depends on species characteristics of the microorganism and nature of the luminescent system.

Key Words: *bacterial luminescence; blood serum; Photobacterium phosphoreum; Photorhabdus luminescens; Escherichia coli*

During the last decade luminescent microorganisms became a popular instrument for the studies of various abiotic media [6,8]. The level of inhibition of bioluminescence of *Photobacterium phosphoreum*, *Vibrio fischeri*, and some other natural luminescent microorganisms is in good correlation with the content of chemical pollutants in the analyzed samples; measurement of luminescence inhibition is a convenient instrument for rapid evaluation of combined effects of all present pollutants [2,10]. Creation of recombinant strains of *Escherichia coli* carrying lux-operons of natural luminescent bacteria [7] maximally simplified the biotesting procedure and made it possible to extend the sphere of applying the bioluminescence phenomenon to studies of human and animal biological fluids [12,13], for which *E. coli* is a permanent symbiont.

We compared the effects of human serum on bioluminescence intensity in some natural and recombinant luminescent bacteria and detected the influence of concentration, temperature, and duration of exposure on these effects.

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MATERIALS AND METHODS

The study was carried out on a natural strain of sea bacterium *Photobacterium phosphoreum* (commercial name Microbiosensor B-17 677f, Institute of Biophysics, Siberian Division of Russian Academy of Sciences), strains of soil bacterium *Photorhabdus luminescens* Zm1 (gracious gift from V. S. Danilov and A. P. Zarubina, M. V. Lomonosov Moscow State University), and 3 recombinant strains of *E. coli*: Z905 (Institute of Biophysics, Siberian Division of Russian Academy of Sciences), Ecolum-5 and Ecolum-8 (M. V. Lomonosov Moscow State University), containing plasmids constructed on the base of pUC18 vector, two former ones carrying genes of the *Photobacterium leiognathi* luminescent system [3] and the latter one including *Photorhabdus luminescens* Zm1 lux-operon [5]. Serum pool from 20 donors was used for the regulation of spontaneous bioluminescence of these microorganisms.

Luminescent microorganisms in concentrations of 10^8-10^9 CFU/ml (50 μl) were mixed with equal volumes of the serum pre-diluted with 0.5% NaCl to the concentrations of 100 to 10% of the initial concentration with a 10% "step". Man-made sea water served as the control for *P. phosphoreum*, 0.85% NaCl for *P.*

0007-4888/04/1383-0276

luminescens and *E. coli*. For experiments at 4°C, 22-24°C, and 37°C all components were pre-cooled or preheated to the needed temperature. The mixtures were exposed during 10, 20, 30, 40, 50, and 60 min, after which the reaction was arrested by adding 10-fold volume of the corresponding saline and the luminescence level was measured using a BLM-8820 bioluminometer (Nauka Firm). Bioluminescence was expressed in percent of the control. The results were processed by the routine variation statistics methods. Analysis of dispersions was used for evaluating the effect of each studied factor on the intensity of bacterial luminescence.

RESULTS

Changes in bioluminescence of *P. phosphoreum* at 22-24°C were biphasic. By the 10th min of contact the presence of 10-80% serum (*vs.* the initial concentration) manifested by stimulation of bioluminescence, maximally surpassing the baseline values (65.0±5.6%) in the presence of the minimum concentrations and gradually passing into the zone of negative values as the percent content of the active agent increased. Prolongation of incubation to 30 min led to a decrease in the absolute level of bioluminescence stimulation and to a decrease in the threshold concentration of the serum (>50%), surpassing which we observed an inhibitory effect, maximally pronounced (-24.2±3.3% of control) in the presence of undiluted serum (Fig. 1) and persisting until the 60th min of the contact. If the experiment was carried out at 4°C, the inhibitory effect of the serum on *P. phosphoreum* luminescence decreased and the zone of luminescence stimulation was

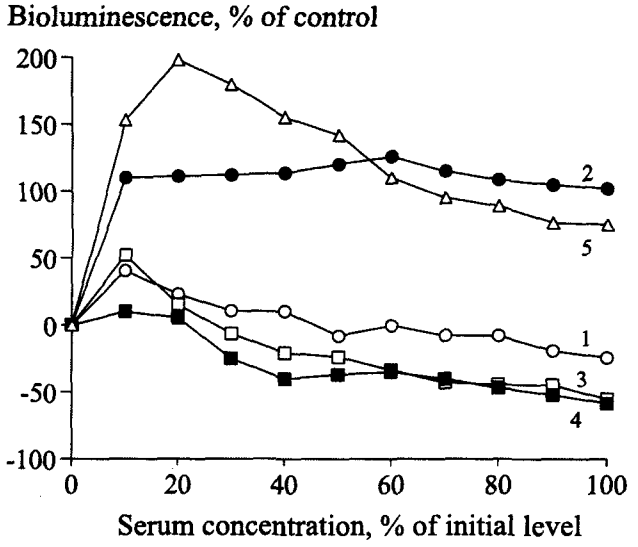

Fig. 1. Strain-specific features of reactions of natural and recombinant luminescent bacteria to blood serum (22-24°C, 30 min). *1*) *P. phosphoreum* B-17 667f; *2*) *P. luminescens* Zm1; *3*) *E. coli* Z905; *4*) *E. coli* Ecolum-5; and *5*) *E. coli* Ecolum-8.

extended. The increase of incubation temperature to 37°C led to a significant inhibition of bioluminescence by the 10th min in the presence of the serum in a wide range of concentrations, which, however, could not be demonstrated over the course of the experiment. Just residual bioluminescence indiscernible from the background was observed in all samples, which was due to the temperature range beyond the optimum for the activity of the *P. phosphoreum* luminescent system [1]; this makes this microorganism an inconvenient object for testing under these conditions.

Experiments on soil bacterium *P. luminescens* Zm1 showed that this microorganism responded by

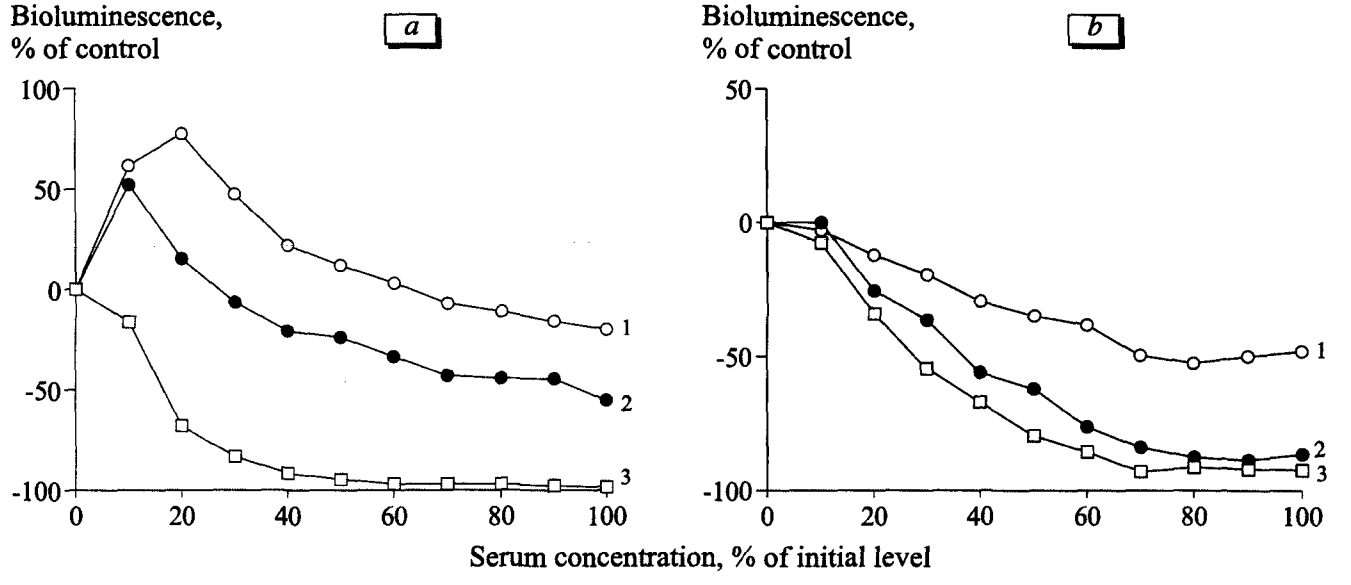

Fig. 2. Effects of thermal conditions (*a*) and duration of exposure (*b*) with the serum on bioluminescence of recombinant *E. coli* strains. *a*) For *E. coli* Z905 at 30-min incubation: *1*) at 4°C; *2*) at 24°C; *3*) at 37°C; *b*) for *E. coli* Ecolum-5 at 37°C: *1*) 10 min; *2*) 30 min; *3*) 60 min.

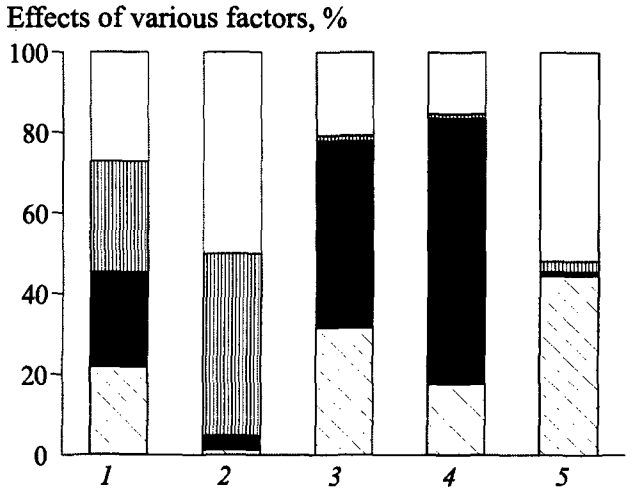

Fig. 3. Effects of some factors (%) on bioluminescence of natural and recombinant luminescent bacteria. *1*) *P. phosphoreum* B-17 667f; *2*) *P. luminescens* Zm1; *3*) *E. coli* Z905; *4*) *E. coli* Ecolum-5; *5*) *E. coli* Ecolum-8. Light bars: effects of unknown factors; vertically hatched bars: effects of duration of exposure; dark bars: effects of temperature of incubation; cross-hatched bars: effects of serum concentration.

stimulation of luminescence to incubation with the blood serum in a wide range of concentrations and different thermal and time regimens. At 22-24°C stimulation of luminescence from 102.0±6.7% to 125.8±5.6% of the initial level was observed in the presence of all tested serum concentrations until the 30th min of exposure (Fig. 1), while inhibition of luminescence was observed only at the late periods of incubation (50-60 min) with the serum in concentrations of 80-100% of the initial and reached -6.6±2.2% during incubation with intact serum. At 4°C the inhibitory effect of the serum on *P. luminescens* luminescence disappeared completely. At 37°C the inhibitory effect developed starting from the 20th min of exposure in the presence of serum in high concentrations, and by the 60th min the values were negative irrespective of the serum dilutions.

In general, *P. luminescens* luminescence was sufficiently resistant to the inhibitory effect of the serum, which could be due to ecology of this microorganism. This hypothesis is based on the fact that during the natural cycle of its existence *P. luminescens* is often exposed to humoral factors of invertebrates' immunity, which have much in common with some serum effector mechanisms eliminating the infective agents in mammals [4]. Hence, *P. luminescens* resistance to the serum can be regarded as a mechanism providing its transition from commencealism (in entomopathogenic nematodes) to parasitism (in insects) [9].

The use of recombinant *E. coli* Z905 strain carrying pPHL7 plasmid with the *P. leiognathi* lux-operon showed the picture largely resembling that observed for *P. phosphoreum*; this can be explained by the presence of common antigenic determinants in these microorganisms and similarity of their cell wall organization [11], effectively damaged during exposure to the sum of serum factors. At 4°C and 22-24°C biphasic changes in the levels of bacterial luminescence were observed: luminescence stimulation in the presence of low concentration of the serum and inhibitory effect of high serum concentrations (Fig. 2, *a*). However, replacement of the test object (natural *P. phosphoreum* strain) with recombinant *E. coli* Z905 strain led to lowering of the threshold concentration of the serum, beyond which the bacterial luminescence was suppressed. At 37°C the pronounced inhibitory effect developed rapidly, reaching by the 30th min of the contact -95.0±4.3% for all serum concentrations >50% of the initial one.

Experiments with *E. coli* Ecolum-5 strain also demonstrated similar response of bacterial luminescence, which can be explained by the identity of the recipient strain (*E. coli* K12 TG1) used for its creation, vector (pUC18), and similarity of cloned *P. leiognathi* luminescence system. At 37°C the intensity of the inhibitory effect increased, while the stimulatory effect of low concentrations of the serum was absent (Fig. 2, *b*).

Analysis of the serum effect on recombinant *E. coli* strain carrying lux-operon of soil *P. luminescens*

TABLE 1. Parameters of Natural and Recombinant Luminescent Bacteria Determining the Prospects of Their Use for Testing Serum Bactericidal Activity

Parameters	*P. phosphoreum* B-17 677f	*P. luminescens* Zm1	E. coli		
			Z905	Ecolum-5	Ecolum-8
Possibility of testing at 37°C	—	+	+	+	+
No zone of luminescence stimulation during incubation with the serum at 37°C	+	—	+	+	—
Significant relationship between level of luminescence inhibition and serum concentration	+	—	+	+	+

Note. «+» yes, «—» no.

Zm1 bacterium (Fig. 1) showed other characteristics of the reaction, differing this strain from other recombinant *E. coli* strains, and consisting in predominant stimulation of bacterial luminescence, as was observed with the donor strain. Significant inhibition of luminescence was observed only at 37°C after long (>30 min) incubation in the presence of high serum concentrations (at least 80%).

Analysis of the intensity of the effects of each of the three factors on the intensity of bioluminescence of natural and recombinant bacteria showed that their sum determined 47.7-84.6% of the modifying effect of the serum on the level of bacterial luminescence (Fig. 3). The effects of factors neglected in this study and presumably connected with organization of the luminescent system, function of bacterial cells, fluctuations in their number in the sample, *etc.*, were the maximum in the natural *P. luminescens* Zm1 strain (50.1%) and the recombinant *E. coli* Ecolum-8 strain created on the base of this strain (52.3%).

Hence, the effect of the duration of exposure was the least, being most significant for natural *P. phosphoreum* B-17 677f and *P. luminescens* Zm1 strains (27.7 and 45.1%, respectively). The thermal factor was more significant for the modifying effect of the blood serum on bioluminescence of *P. phosphoreum* B-17 677f and two recombinant *E. coli* strains with cloned *P. leiognathi* lux-operons (23.4-65.9%). Serum concentration was the most significant factor determining the direction and intensity of the effect. It determined 17.6-44.1% ($P<0.05$) variability of bioluminescence in 4 of the 5 studied natural and recombinant strains (except *P. luminescens* Zm1). This latter fact, together with the possibility of carrying out experiments at 37°C and the absence of the zone of serum stimulatory effect on bacterial luminescence under these thermal conditions, can be regarded as a criterium for the selection of luminescent bacteria to be used for testing the serum antibacterial effect (Table 1). By the sum of our evaluations, the use of recombinant *E. coli* Z905 and Ecolum-5 strains seems to be the most promising, because these strains give the most stable clearly interpreted results.

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