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EXPERIMENTAL ARTICLES

The Effect of Red and Infrared Light on the Growth of *Escherichia coli* and the Production of the Recombinant Protein Barstar

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Abstract—Incoherent red and infrared low-intensity light enhanced the growth of the auxotrophic strain *Escherichia coli* AD494(DE3)pLysS and the production of the recombinant polypeptide barstar. Illumination also stimulated the growth of nonrecombinant *E. coli* cells.

Key words: *Escherichia coli*, low-intensity radiation, barstar, genetic transformation.

Physical fields, including low-intensity radiation of various wavelengths, induce diverse effects in living organisms [1]. In particular, low-intensity red and infrared light stimulates the growth of the bacteria *Escherichia coli* and *Pseudomonas fluorescens* [2, 3] and shortens their lag phase [4]. In his recent work, Karu discussed various mechanisms that may be responsible for the stimulating effect of light on *E. coli* cells [5].

The aim of this work was to study the effect of red and infrared light on the growth of the auxotrophic strain *E. coli* AD494(DE3)pLysS and the production of the recombinant polypeptide barstar.

MATERIALS AND METHODS

The strain *Escherichia coli* AD494(DE3)pLysS with the genotype Δ*ara leu7967* Δ*lacX74* Δ*phoAPvuII phoR* Δ*malF3* F'[lac^+($lacI^q$)*pro*] *trxB*::*kan* (DE3), (Cm^R) was purchased from Novagen (United States). The strain is auxotrophic for leucine and is characterized by low viability. The strain was grown in M9 medium [6] supplemented with 10 ml of a 20% glucose solution, 1 ml of 1 M $MgSO_4$, 0.1 ml of 1 M $CaCl_2$, and 20 primary amino acids in amounts of 50 μg per l. Selective conditions were created by adding ampicillin at a final concentration of 100 μg/ml. Cells were transformed in the presence of $CaCl_2$ [6] with the vector plasmid pGEMEX-1/Bst carrying the gene of barstar (a polypeptide inhibitor of RNases [7]) and the gene of ampicillin resistance. The vector plasmid was obtained from A. Shul'ga, Institute of Molecular Biology, Moscow. It operates in the pET expression system, whose mechanism of action was described by Mierendorf and Yeager [8]. Incoherent light with wavelengths of 600 and 900 nm was generated using a 20-mW device for phototherapy purchased from ZAO Medical and Ecological Center Dyuny (Tomsk, Russia). The radiation dose was 4 kJ/m^2, which is optimal for the spectral region studied [9]. Irradiated recombinant cells were placed in the ampicillin-containing selective medium that favors the expression of barstar. Nonrecombinant cells, either irradiated or not, were grown in the same medium but without ampicillin. The initial optical density (OD_{600}) of cultures was 0.1. Bacteria were grown at 37°C on a shaker (120 rpm) for 20 h. Culture growth was monitored by measuring, at 2-h intervals, the optical density of cultures at 600 nm using a Specord M-40 spectrophotometer.

The electrophoresis of proteins was carried out in 12% polyacrylamide gel [10].

The results were statistically processed using Origin 6.0 software. The data presented in the paper are the means of 2–3 replicated measurements.

RESULTS

The effect of illumination on the growth of the recombinant and nonrecombinant cells of strain AD494(DE3)pLysS is shown in Fig. 1. Data on the specific growth rate and the biomass (these parameters were calculated from optical density data as described by Schlegel [11]) are presented in the table. As can be seen from Fig. 1 and the table, both illuminated and unilluminated recombinant cells grew more poorly than nonrecombinant cells. Illumination stimulated the growth of recombinant cells and, to a lesser degree,

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The effect of illumination on the growth parameters of the control *E. coli* cells and those subjected to (1) transformation, (2) illumination, and (3) combined transformation and illumination

Growth parameter	Variant of cells			
	Control	1	2	3
Specific growth rate, h^{-1}	0.26 ± 0.012	0.12 ± 0.082	0.3 ± 0.059	0.21 ± 0.044
Biomass, OD unit	0.67 ± 0.037	0.21 ± 0.098	0.77 ± 0.039	0.46 ± 0.064

the growth of nonrecombinant cells (Fig. 1, curves *2* and *4*). This is also evident from the data presented in the table.

Electrophoretic analysis showed that illumination also favored the biosynthesis of barstar in recombinant cells (Fig. 2).

DISCUSSION

It is known that radiation of different wavelengths can induce various physical and chemical changes in photoacceptors and the components of respiratory chains, thereby modifying the cell homeostasis. The photoinduced shift of the redox state of cells toward oxidation stimulates their metabolic activity, whereas the shift toward reduction inhibits it [5]. In particular, red and infrared light shifts the redox state of cells toward oxidation and enhances the synthesis of ATP and DNA [12, 13]. This can explain the photoinduced stimulation of the growth of recombinant and nonrecombinant cells and the enhancement of barstar synthesis in the recombinant cells observed in our experiments.

The poor growth of recombinant cells can be related to the synthesis of barstar, since almost all cell resources are involved in this synthesis (the production of barstar may reach 30–50% of the total protein content of the recombinant cells [8]). This impairs the normal functioning of cells, in particular, their growth (Fig. 1, curves *1* and *3*).

Barstar is a polypeptide inhibitor of some bacterial ribonucleases (such as barnase in *Bacillus amiloliquefaciens* and its analogue binase in *Bacillus intermedius*) and, hence, may suppress the RNases of *E. coli* cells or be toxic to them in some way. The different responses of recombinant and nonrecombinant cells to equal illumination doses can be explained by the fact that barstar in the recombinant cells may alter normal protein–protein interactions and impair the functioning of photoacceptors (in *E. coli*, the oxidase complexes *bo* and *bd* [14]).

Thus, red and infrared light stimulates the growth of recombinant and nonrecombinant cells and enhances the synthesis of barstar in the recombinant cells, which can be accounted for by photoinduced alterations in the physiological state of these cells.

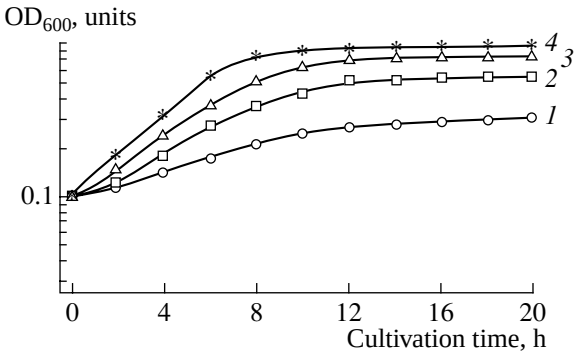

Fig. 1. Growth of the auxotrophic *E. coli* AD494(DE3)pLysS strain: (*1*) unilluminated recombinant cells; (*2*) illuminated recombinant cells; (*3*) control (unilluminated nonrecombinant) cells; and (*4*) illuminated nonrecombinant cells.

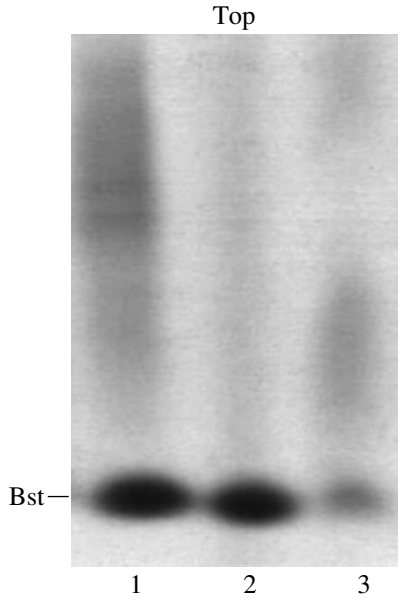

Fig. 2. Electrophoresis in 12% polyacrylamide gel. Lanes: 1, lysate of illuminated recombinant cells; 2, purified barstar; and 3, lysate of unilluminated recombinant cells.

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