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Culture-to-culture physical interactions causes the alteration in red and infrared light stimulation of *Escherichia coli* growth rate

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Escherichia coli MC1061cells were irradiated at 660 and 900 nm, incubated in M9 and LB media with the use of a specially constructed device, and assayed for growth rate. There was a reduction of growth rate stimulation when the irradiated culture was cultivated jointly with the non-irradiated one. In the same time, the irradiated culture extended the invigorate effect on the non-irradiated one. It is proposed that the effects observed were mediated by culture-to-culture physical interactions.

Key words: Growth rate, physical interactions, *Escherichia coli*

Light in the red and infrared regions of the spectrum has a variety of positive effects on bacterial cells. Several experiments have been reported with evidence that the irradiation with red and infrared light can accelerate the rate of division and growth of *Escherichia coli* cultures [1]. The mechanisms for bacterial photo-induced biostimulation have been discussed [2]. Yet, little is known of the factors, which are capable of modifying the growth-stimulating effects of red and infrared light on bacteria. At the same time, there is increasing evidence for the phenomenon of physically mediated communication in bacterial cultures for some events, including cell division [3], adaptation of microorganisms to stress conditions [4,5], adhesive capabilities of cells [6] and regulation of light emission [7]. Therefore, the non-irradiated bacterial culture (which were cultured jointly with the irradiated one) could modify the growth-stimulating effect of red and infrared light.

The aim of the present work was to study the red and infrared light effects on the *E. coli* growth rate in the conditions of distant interactions of irradiated and non-irradiated bacterial cultures.

Materials and Methods

Bacterial strains and culture media

The *E. coli* MC1061 strain (ΔacX74 Δara-leu galK strA hsdR) was obtained from Institute of Biochemistry and Physiology of Microorganisms (Puschino, Russia). Two different culture media were used: LB (1% tryptone, 0.5% yeast extract, and 1% NaCl) and M9 (each liter containing: 6 g Na_2HPO_4, 3 g KH_2PO_4, 0.5 g NaCl, and 1 g NH4Cl) supplemented with 0.2% glucose [8]. For both media, the pH was adjusted to 7.5.

Radiation source and irradiation procedure

Radiation at 660 and 900 nm was obtained from an apparatus for phototherapy "Duna-T," radiation power was 20 mW (Tomsk, Russia). The fluence used (6 kJ/m^2) was previously found to be optimal for this spectral region [1]. Before the experiments were done, *E. coli* MC1061, which had been stored in a glycerol stock (10% w/v) at –20°C, was pre-grown at 37°C in the stock solution for 24 h and subcultured twice at 37°C for 12 h after transferring to culture medium. The one part of the pre-culture was prepared for irradiation as it was described in [1]. The other part of the pre-culture was centrifuged at 5000 g during 5 min and then re-suspended to the necessary optical density in fresh nutrient medium.

Experiments were performed with the use of a specially-constructed device (Fig. 1), made from usual (UV-opaque) glass "Pyrex-P15." The device was a cylinder that was separated into equal compartments by a glass window. The glass separation between the compartments was watertight. The ends of both cylindrical compartments were closed using a screw-cap with a rubber septum. The volume of each compartment was 20 mL. A modified device with an opaque glass window between compartments was also made. The opaque glass was nontransparent for UV and visible light. In order to remove the influence of natural light, all the devices were enveloped in aluminum foil.

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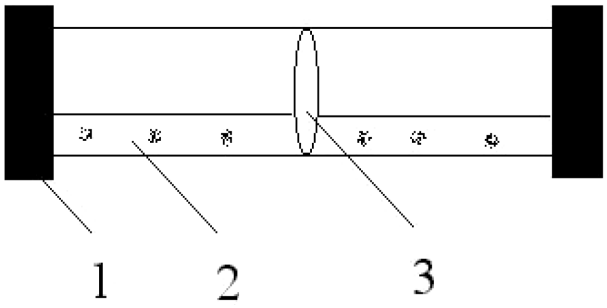

Fig. 1. Experimental device for investigation of culture-to-culture physical interactions between bacterial cells.
1 Screw-cap of cylinder. 2 Medium with growing cells. 3 Glass window (opaque and clear).

Growth conditions and experimental design

All incubations were done at 37°C in a shaking incubator (120 rpm). Cells were grown in 10 mL of M9 or LB nutrient media in adjacent parts of the device. The culture under study were referred to as Control (non-irradiated alone), Culture 1 (irradiated alone), Culture 2 (irradiated and adjacent to non-irradiated), Culture 3 (non-irradiated and adjacent to irradiated). The mean initial optical density values for cells were between 0.095-0.100.

Samples (0.5 mL) for optical density (OD) measurements were taken from the compartments of the device by using a sterile syringe thorough the rubber septum. Growth was monitored using light scattering by measuring the OD_{600} value, which was measured in quartz cuvettes and a 0.2-mm light path with the use of Specord M40 Spectrophotometer. After the OD_{600} readings had been taken, the samples were discarded. To estimate the growth rate, the equation proposed by Shlegel was applied [9].

Statistics

For each culture condition, the values of the growth rate obtained in 10 replicates were analyzed for goodness of fit to normal distributions by using the Shapiro-Wilk's (W) test. Pairwise comparisons between growth conditions were made for mean values of the growth rate using the one-sided Student *t* test [10]. Calculations were made using the STATISTICA software for Windows (release 5.0).

Results and Discussion

The mean values for the growth rate (h^{-1}) calculated and their standard deviations are presented in Table 1. The application of the Shapiro-Wilk's (W) test allowed me to conclude that the data obtained followed a normal distribution (Table 1). Therefore, a Student *t* test was employed to test whether the population means were equal (Table 2).

It is clear from Table 1 that the growth-stimulant effects of red and infrared radiation decreased when Culture 2 (irradiated and adjacent to non-irradiated) was cultivated jointly with Culture 3 (non-irradiated and adjacent to irradiated). The phenomenon above was developed for the both growth media used in the research. Furthermore, there was the maximal reduction (10.65%, $p<0.05$) of growth rate stimulation due to conjoint LB cultivation of Culture 2 (irradiated and adjacent to non-irradiated) and Culture 3 (non-irradiated and adjacent to irradiated). When the cultures above were jointly cultivated in M9 medium, the reduction of growth stimulation was less (2.18%, $p<0.05$).

On the other hand, the values for the growth rate of Culture 3 (non-irradiated and adjacent to irradiated) grown jointly with Culture 3 (irradiated and adjacent to non-irradiated) were greater than those of Control. The stimulation of growth rate were at 5.46% ($p<0.05$) and 2.29% ($p<0.01$) (in M9 and LB, respectively).

Table 1. Estimation of growth rate (h-1) of E. coli MC1061 cultures cultivated together in M9 and LB media and the values of Shapiro-Wilk's test for goodness of fit to normal distribution for E. coli MC1061growth rate

Type of growth medium	Type of culture			
	Control[a]	Culture 1[b]	Culture 2[c]	Culture 3[d]
M9	0.549 ± 0.019	0.599 ± 0.023	0.586 ± 0.021	0.579 ± 0.045
	0.924[e]	0.969[e]	0.906[e]	0.933[e]
LB	0.786 ± 0.031	0.936 ± 0.049	0.839 ± 0.051	0.804 ± 0.041
	0.995[e]	0.948[e]	0.944[e]	0.971[e]

[a]Non-irradiated alone.
[b]Irradiated alone.
[c]Irradiated and adjacent to non-irradiated.
[d]Non-irradiated and adjacent to irradiated.
[e]Values of Shapiro-Wilk's test.

Table 2. Values of Student *t* test for growth rate of *E. coli* MC1061

Type of growth medium	Type of comparison			
	Control[a] vs Culture 1[b]	Control vs Culture 2[c]	Control vs Culture 3[d]	Culture 1 vs Culture 2
M9	21.77 (p<0.001)	5.81 (p<0.010)	2.46 (p<0.050)	2.71 (p<0.050)
LB	7.93 (p<0.001)	3.09 (p<0.050)	2.62 (p<0.050)	3.55 (p<0.010)

[a]Non-irradiated alone,
[b]Irradiated alone,
[c]Irradiated and adjacent to non-irradiated,
[d]Non-irradiated and adjacent to irradiated,
Values of Shapiro-Wilk's test.

It is important to note that there were no any effects observed during joint cultivation of Culture 2 and Culture 3 when the device with the opaque glass window between the adjacent compartments was used.

Since the chemical transmittance between the cultures under study was eliminated, it is reasonable to propose that the effects described here may point to the ability of culture-to-culture interaction via physical signals. This finding indicated that irradiated and non-irradiated cultures altered their growth rate during conjoint cultivation begged the question for the nature of these signals.

Many investigations demonstrated that the interaction described above was mediated by the transfer of UV signals [11,12] rather than by visible radiation. In the present study, it could not be explained by the cultures interacting in the UV range of the spectrum because the devices used to culture the bacteria were made from glass, which absorbs UV radiation.

Recently, Matsuhashi and coworkers showed that the bacterium *Bacillus carboniphilus* used a physical signal for inducing the germination spores under severe conditions, and for modulating the sensitivity of the organism to antibiotics [4,5]. They postulated the sonic nature of the signal. However, in this study, there were no statistically significant effects during cultivation of cultures in the device with an opaque glass window between the adjacent compartments compared to control experiments. This fact excludes the sonic nature of the signal in the present work. Thus, it may be proposed that the most probable candidates for signal carriers are visible and/or near-IR radiation.

The ability of near-IR radiation to be a signal carrier has been demonstrated in the experiments with BHK cells [13]. Albrecht-Buehler showed that BHK cells on one face of the thin-glass window of the device used were able to respond to the orientation of other BHK cells on the other side of the device above. Therefore, he concluded that cells normally emit pulsating infrared signals and that the described IR "vision" is used by cells to detect each other at a distance. The same phenomenon has been described for 3T3 cells [14], *Rodospirillum rubrum* cells [15] and live mammalian cells [16]. It is possible to assume that the *E. coli* cells studied here also possess the ability to detect each other at a distance and alter their behavior (in particular, the growth rate) using near-IR signals.

The ability to interact via visible light has been demonstrated for *Pseudomonas fluorescens* cells [6]. Nikolaev showed convincingly that adjacent cultivation of bacterial cultures in an ordinary glass device "flask-in-flask" resulted in significant reduction of adhesive capabilities of cells. Thus, the literature data [6,7,17] suggest that the likely explanation for the phenomenon described in the present work involves the processing of visible light signals (and/or near-IR signals) by the *E. coli* cells. Whatever the explanation, the probable source of photon radiation from cells needs to be established.

Investigating the influences of the red and infrared light on the of *E. coli* growth rate without any distant interactions between bacterial cells was not the aim of the present work. Therefore, other factors that influence the character of growth rate alterations need to be considered. It is well known that the light induced emission from cells is more intense than a spontaneous one [18]. Probably, the non-irradiated cells might detect the intensive photon flux from the irradiated. The intensive photo-induced light flux might also be reverberated from the aluminum foil covered the device. Anyway, the non-irradiated cells could send the growth-inhibiting signals to the adjacent compartment of the device used that caused the reduction of growth rate. Contrariwise, the irradiated culture could serve as a sender of growth-stimulating signals. However, the aforementioned phenomena should be investigated thoroughly in prospective studies.

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