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Studies on distant regulation of bacterial growth and light emission

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Reciprocal interactions of two *Escherichia coli* MC1061 cultures separated by a glass window were investigated. The growth parameters and light emission from these cultures were analysed. A link between light emission and the growth parameters was observed.

INTRODUCTION

It is now commonly accepted that all living organisms emit a weak radiation over a wide spectral range. Some authors consider this emission an indicator of a fundamental regulatory role played by photons and excited molecular states in cells, tissues and even the whole organism (Chang *et al.*, 1998). Others insist that the observed emissions represent yet another kind of radiation resulting mostly from oxidation reactions (Slawinska & Slawinski, 1983). The main question concerning emission now is related to whether biological radiation carries information pertaining to intercellular communication and cell growth.

Up until now, progress in understanding the intercellular interactions of bacteria has been connected with investigations of prokaryotic signalling molecules (Kaprelyants & Kell, 1996). However, there is increasing evidence for the widespread importance of physically mediated communication in bacterial cultures for some events, including cell division (Nikolaev, 1992), adaptation of micro-organisms to stress conditions (Matsuhashi *et al.*, 1996) and adhesive capabilities of cells (Nikolaev, 2000).

In this paper, I report my observations on (i) the regulation of *Escherichia coli* MC1061 growth and (ii) a weak light emission in the absence of any chemical or mechanical contacts between the cultures under study.

METHODS

Bacterial culture. *E. coli* MC1061 (Δ*lacX74* Δ*ara–leu galK strA hsdR*) was obtained from the Institute of Biochemistry and Physiology of Microorganisms (Puschino, Russia).

Experimental set-up. Experiments were performed with the use of a specially constructed device (Fig. 1), which was made from usual glass Pyrex-P15. Fig. 2 presents the light absorption spectrum of the glass. 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 40 ml. A modified device with an opaque glass window between compartments was also made. The opaque glass was non-transparent for UV and visible light. In order to remove the influence of natural light, all the devices were enveloped in aluminium foil.

Culture media. Two different culture media were used: LB (1 % tryptone; 0·5 % yeast extract; 1 % NaCl) and M9 (6 g Na_2HPO_4 l^{-1}; 3 g KH_2PO_4 l^{-1}; 0·5 g NaCl l^{-1}; 1 g NH_4Cl l^{-1}) supplemented with 0·2 % glucose (Sambrook *et al.*, 1989). For both media, the pH was adjusted to 7·5.

Inoculum build-up. Before the experiments were done, *E. coli* MC1061, which had been stored in a 10 % (w/v) glycerol stock at

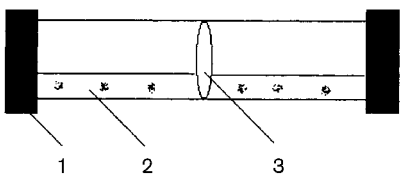

Fig. 1. Experimental set-up for investigation of distant interactions between bacterial cells. 1, Screw-cap of cylinder; 2, medium with growing cells; 3, glass window (opaque and clear).

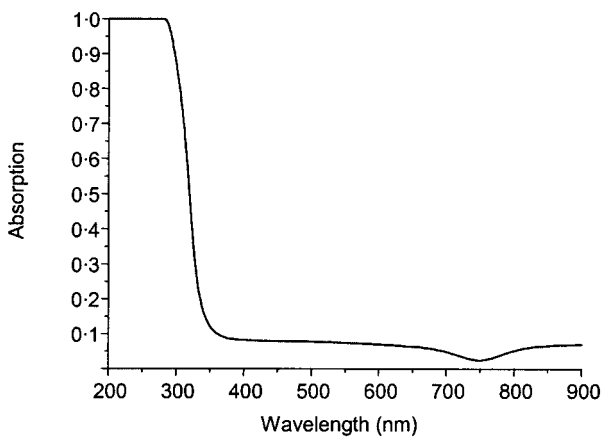

Fig. 2. Light absorption spectrum of Pyrex-P15 glass.

−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 transfer to culture media. The last pre-culture was centrifuged at 5000 ***g*** for 5 min and then resuspended to the necessary optical density in fresh nutrient medium (explanations are below).

Growth conditions and experimental design. All incubations were done at 37 °C in a shaking incubator (120 r.p.m.). Cells were grown in 20 ml nutrient medium (LB and M9 were used) in adjacent parts of the device. From hereon the parts of the device are referred to as Culture Left (adjacent to Right), Culture Right (adjacent to Left) and Culture Left Alone (Control). The mean initial optical density values for cells were between 0·095 and 0·1.

Monitoring growth. Samples (1 ml) for optical density measurements were taken from the compartments of the device by using a sterile syringe through the rubber septum. Growth was monitored using light scattering by measuring the OD_{600} value, which was measured in quartz cuvettes and a 10-mm light path with the use of a Specord M40 spectrophotometer. After the OD_{600} readings had been taken, the samples were discarded. To estimate the growth parameters, the following equation (Zwietering *et al.*, 1990) was applied:

$$D_t = D_0 + A \exp\{-\exp[(\mu e/A)(\lambda - t) + 1]\}$$

where D_t is the optical density at time t, t is the time of growth (h), D_0 is the optical density at $t=0$, A is the increase in optical density between D_0 and OD_{max}, μ is the maximum growth rate (h^{-1}), λ is the duration of the lag phase (h), and e is the base of the Napierian logarithm (2·718281828).

Statistics. For each culture condition, the values of the growth parameters obtained in 14 replicates were analysed for goodness of fit to normal distributions by using the Kolmogorov–Smirnov test. Pairwise comparisons between growth conditions were made for mean values of the different growth parameters using the one-sided Student's *t*-test (Sokal & Rohlf, 1969). Calculations were made using the STATISTICA software for Windows (release 5.0).

Spectral measurements. The radiation from *E. coli* MC1061 was measured using two identical photon counting machines that used the FEU-69 photomutliplier tube, which was sensitive in the 450–800 nm range. Spectra were obtained from 5 ml samples of cultures grown at 37 °C. The spectra from samples taken were scanned simultaneously. In other words, it was possible to fix the emission from jointly cultivated cultures. Spectral measurements were repeated three times.

RESULTS

The mean values for the growth parameters calculated and their standard deviations are presented in Table 1. Primarily, it is necessary to report on all statistical procedures used here. The application of the Kolmogorov–Smirnov test allowed me to conclude that the data obtained followed a normal distribution (Table 2). Therefore, a

Table 1. Estimation of growth parameters of *E. coli* MC1061 cultures cultivated together in M9 and LB media

Type of culture	Type of growth medium	Growth parameters			
		Duration of lag phase (h)	Growth rate (h^{-1})	Generation time (h)	Harvest (OD units)
Culture Left (adjacent to Right)	M9	0·899 ± 0·151	0·567 ± 0·027	1·225 ± 0·055	0·755 ± 0·019
	LB	0·274 ± 0·295	0·794 ± 0·04	0·874 ± 0·044	1·058 ± 0·043
Culture Right (adjacent to Left)	M9	0·871 ± 0·229	0·569 ± 0·044	1·225 ± 0·091	0·753 ± 0·014
	LB	0·366 ± 0·148	0·802 ± 0·034	0·866 ± 0·037	1·07 ± 0·043
Culture Left Alone (Control)	M9	0·643 ± 0·238	0·513 ± 0·035	1·357 ± 0·088	0·761 ± 0·026
	LB	0·345 ± 0·171	0·769 ± 0·032	0·902 ± 0·037	1·196 ± 0·04

Table 2. Values of Kolmogorov–Smirnov test for goodness of fit to normal distribution for different growth parameters

Since the Kolmogorov–Smirnov test values are less than the critical ones (the critical value at $P=0·05$ is 0·895), one can conclude that the data correspond to normal distribution. Optical density was measured at 600 nm.

Type of culture	Type of growth medium	Growth parameters			
		Duration of lag phase	Growth rate	Generation time	Harvest
Culture Left (adjacent to Right)	M9	0·134	0·192	0·187	0·131
	LB	0·139	0·069	0·082	0·188
Culture Right (adjacent to Left)	M9	0·134	0·168	0·141	0·102
	LB	0·176	0·139	0·149	0·155
Culture Left Alone (Control)	M9	0·161	0·172	0·149	0·188
	LB	0·175	0·217	0·206	0·2

Table 3. Values of Student's *t*-test for growth parameters

Optical density was measured at 600 nm.

Type of comparison	Type of growth medium	*t*-Test values for different growth indexes			
		Duration of lag phase	Growth rate	Generation time	Harvest
Culture Left to Control	M9	3·401 ($P<0·01$)	3·605 ($P<0·01$)	3·744 ($P<0·01$)	1·019 (NS)
	LB	1·289 (NS)	2·239 ($P<0·05$)	2·189 ($P<0·05$)	9·416 ($P<0·001$)
Culture Right to Control	M9	4·138 ($P<0·01$)	5·30 ($P<0·001$)	5·213 ($P<0·001$)	0·898 (NS)
	LB	0·624 (NS)	2·660 ($P<0·05$)	2·683 ($P<0·05$)	7·751 ($P<0·001$)
Culture Left to Culture Right	M9	0·509 (NS)	0·146 (NS)	0·005 (NS)	0·223 (NS)
	LB	1·449 (NS)	1·082 (NS)	1·178 (NS)	0·911 (NS)

NS, Effect not statistically significant.

Student's *t*-test was employed to test whether the population means were equal (Table 3).

Duration of the lag phase. As shown in Table 1, the values for the duration of the lag phase of bacteria grown in M9 medium were greater than those of the control ($P<0·01$). There were no statistically significant differences in the duration of lag phase when LB medium was used.

Growth rate. In M9 medium ($P<0·01$ for Culture Left; $P<0·001$ for Culture Right) and LB medium ($P<0·05$), the values for the growth rate of the cultures cultivated jointly in the device were greater than the control ones.

Generation time. All cultures under study had a shorter generation time when compared with the control sample ($P<0·01$ for Culture Left, and $P<0·001$ for Culture Right in M9 and $P<0·05$ in LB, respectively).

Harvest of cells. There was no statistically significant difference between cells grown in M9 medium and the control samples for this parameter. When cultures were grown in LB medium, the harvest values were less than the control ones ($P<0·001$).

It is important to note that there was no statistically significant difference in the values for the growth parameters of the collated rows Culture Left (adjacent to Right) to Culture Right (adjacent to Left) in all the experiments. Also, there were no effects observed during joint cultivation of the cultures when the device with the opaque glass window between the adjacent compartments was used.

The weak visible region radiation from *E. coli* MC1061 is shown in Figs 3–5. It is clear from the figures that the spectral composition is different for cultures in different growth conditions and nutrient media. For example, Fig. 3(a) shows that during lag phase the emission intensities of jointly grown M9 cultures are identical. The intensities in the region from 450 to 530 nm were much lower than the control ones but the characteristic peaks were preserved. It is important to note that the alteration in the intensity of the peaks was observed at about 530–680 nm. The emission intensities of cultures under study were also

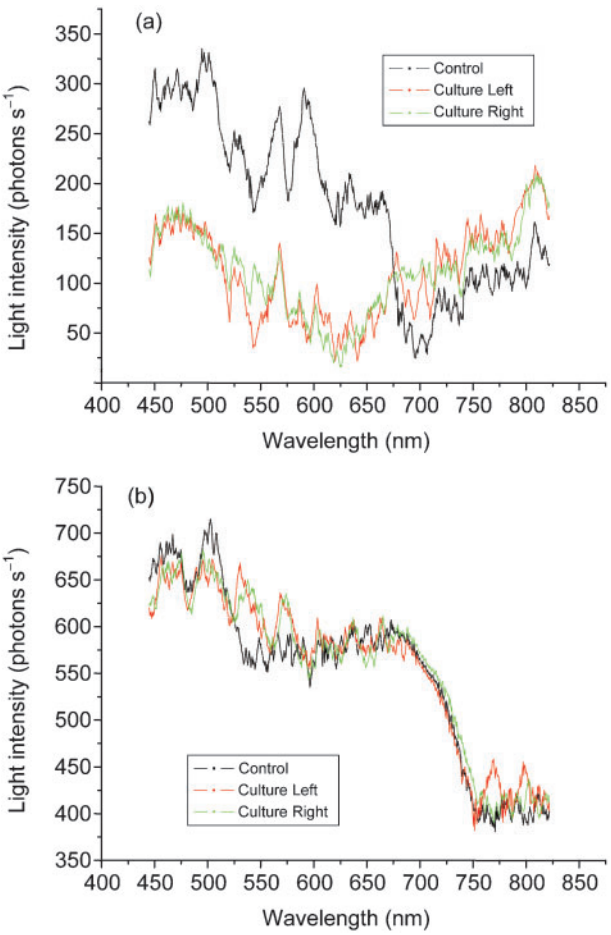

Fig. 3. Radiation spectra of the *E. coli* MC1061 during the lag phase of growth. (a) Cultivation in M9 medium; (b) cultivation in LB medium.

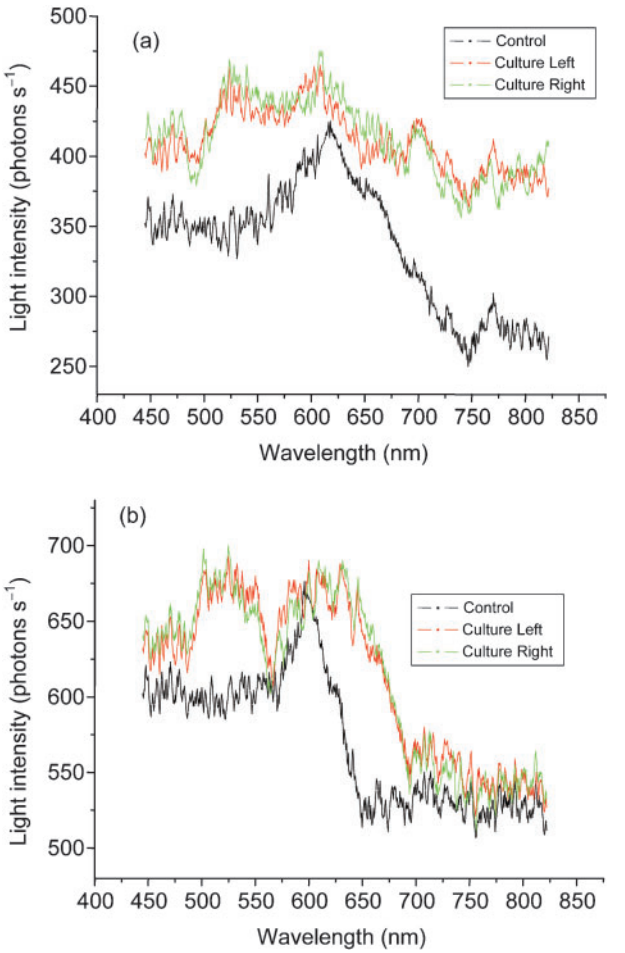

Fig. 4. Radiation spectra of the *E. coli* MC1061 during the exponential phase of growth. (a) Cultivation in M9 medium; (b) cultivation in LB medium.

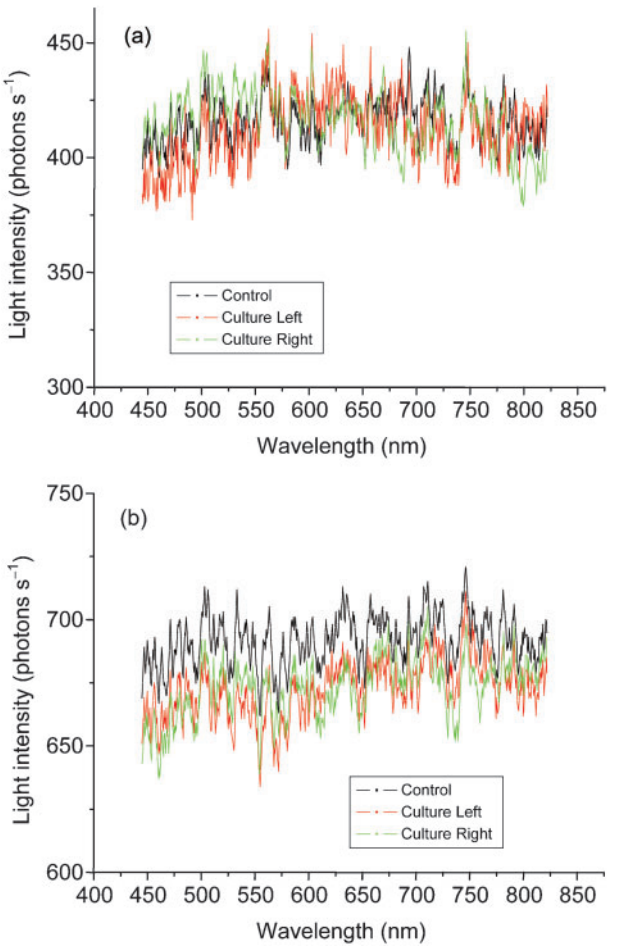

Fig. 5. Radiation spectra of the *E. coli* MC1061 during the stationary phase of growth. (a) Cultivation in M9 medium; (b) cultivation in LB medium.

lower than control values. Between 680 and 800 nm the emission of cells was comparable to the control level. There were no differences in the emission intensities of LB-grown cultures (control as well as cultures under study) during the lag phase of growth (Fig. 3b). There were no alterations in the intensity of the peak composition, apart from Culture Left (adjacent to Right); Culture Left (adjacent to Right) shows modified peaks at 530, 770 and 798 nm.

The spectral distributions of the exponential-phase emissions are shown in Fig. 4 for the two different nutrient media used in this study. In general, when grown in M9 (Fig. 4a) and LB (Fig. 4b), the emissions from the cultures of interest were higher in comparison with the control values. The most noticeable differences in the emission intensities of the cultures under study were at about 520 nm and 700–820 nm (in M9 medium) and at 500–550 nm (in LB medium).

In contrast to the exponential-phase emissions, the spectral distributions of the stationary-phase emissions varied with culture media. Thus, there were no differences in the emission intensities of M9-grown cultures (Fig. 5a). LB-grown cultures showed lower emission intensities than control cultures (Fig. 5b). The sequences of the intensity peaks were identical to the control ones.

When the device with the opaque glass window between the adjacent compartments was used, there were no differences in the emission intensities of the control and cultures of interest.

DISCUSSION

Research into the physically mediated interactions of microbial cultures started immediately after the discovery of mitogenetic radiation (MR) by Alexander Gurvitch in the 1920s (Gurvitch, 1926). His observation stimulated early research, which led to over 500 publications on the ability of MR to stimulate cell division (Rahn, 1936). However, the experimental design of these early works did not exclude the

possibility of metabolite exchange between the cultures under study.

In the present work, the chemical transmittance between cultures of *E. coli* MC1061was eliminated. Therefore, it is reasonable to propose that the results described above occurred due to an electromagnetic interaction between the two cultures. I am addressing the question of what is the nature of the microbial interactions observed. There is a lot of work that states that the interaction described above was mediated by the transfer of UV signals (Gurvitch, 1926; Konev, 1967) rather than by visible radiation. In the present study, one of my conclusions is that the results obtained here can 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 (Fig. 2).

In 1995, Matsuhashi and co-workers showed that the bacterium *Bacillus carboniphilus* used a physical signal for inducing the germination of spores under severe conditions, and for modulating the sensitivity of the organism to antibiotics (Matsuhashi *et al.*, 1995, 1996). They postulated the sonic nature of the signal. However, in my case, 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 IR radiation to be a signal carrier has been demonstrated in experiments with BHK cells (Albrecht-Buehler, 1992). 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. 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 (Albrecht-Buehler, 1991), *Rhodospirillum rubrum* cells (Albrecht-Buehler, 1997) and live mammalian cells (Albrecht-Buehler, 2000). It is possible that the *E. coli* cells studied here also possess the ability to detect each other at a distance and alter their behaviour (in particular, their growth and emission of their own weak radiation) using IR signals.

The ability to interact via visible light has been demonstrated for *Pseudomonas fluorescens* cells (Nikolaev, 2000). Nikolaev showed convincingly that adjacent cultivation of bacterial cultures in an ordinary glass device 'flask-in-flask' resulted in a significant reduction of the adhesive capabilities of cells. Thus, the literature data (Tilbary & Quickenden, 1988; Nikolaev, 2000) and the radiation spectra presented in Figs 3–5 suggest that the most likely explanation for the phenomenon described in the present work involves the processing of visible light signals (and/or IR signals) by the *E. coli* cells. Whatever the explanation, the probable source of photon radiation from cells needs to be considered.

While there is now agreement about the radiation capacities of bacterial cells, no agreement has been achieved in the interpretation of these phenomena. Most researchers argue that weak radiation appears to be due to excited carbonyl groups and/or excited singlet oxygen dimers arising from lipid peroxidation, which are, in turn, associated with an increase in various reactive oxygen species such as superoxide anions, hydrogen peroxide, hydroxyl radicals and singlet oxygen (Slawinska & Slawinski, 1983). However, a preliminary treatment of the cultures under study with laser irradiation (it is well known that laser irradiation decreases the level of radical reactions) resulted in conservation of the interaction phenomenon in the *E. coli* cultures (M. V. Trushin, unpublished data). Hence, I conclude that the spontaneous radiation due to radical reactions can not be considered an explanation of distant interactions between the cultures of interest. Therefore, I favour the alternative hypothesis which states that weak radiation is the mark of an endogenous electromagnetic field pervading the entire organism, which may act as both sender and receiver of the photons that are the 'electromagnetic bioinformation' used in regulating life processes (Popp *et al.*, 1992).

Other phenomena that influence the character of interactions between cultures need to be considered carefully. It is evident that a single cell within a culture could be a source of weak radiation. But, probably, the integral radiation emitted by the culture is not a result of the simple summation of the radiation from all its single cells. This was partly confirmed by measurements of *E. coli* light emission. For example, at about 620 nm the light intensities of emission of M9-grown control cultures were, in general, equal during the exponential and stationary phases of growth whereas the numbers of cells in the cultures were quite different. Hence, the effect of culture density on radiation seems to be non-linear and should be explored in the future. Concerning the distance as well as the surface of photon exchanges between adjacently grown cultures, it should be noted that these were constant in the experiments conducted here. Investigating the influences of the aforementioned factors on the character of the distant interactions between bacterial cells was not the aim of the present work, and these should be clarified in future studies.

Thus, taken together my findings may be interpreted as evidence that the cultures of *E. coli* are able to interact at a distance via physical fields. The alteration of bacterial growth and the synchronization of light emission of adjacent cultures were the main observations supporting this statement. In the present work, the signal was not UV light or sonic. However, whether the signal belongs to the visible or IR region of light should be explored: the source of radiation should also be investigated.

I put my results on record in the hope that they will stimulate further research into this elusive phenomenon.

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