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Light-mediated "conversation" among microorganisms

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Summary

Light emitted from a wide variety of microorganisms was considered previously as a waste product. However, it is becoming apparent that it might be involved in microbial communication. This paper presents information on such a novel mode of communication in different microorganisms.

Introduction

The phenomenon of light emission occurs in many species of microorganisms, including the symbiotic, saprophytic, parasitic and free-living ones (Meighen, 1994). While the biochemical and biophysical mechanisms of light emission are under extensive investigation (Tilbury, 1992; Chang et al., 1998; Rees, 1998; Popp et al., 2002), the biological purport of this phenomenon remains unclear. A possible answer to the question above is that the light emitted from microorganisms may be used for intercellular communication.

Needless to say microbes survey their environment and react accordingly by either signalling to members of their own species to co-ordinate vital functions, or by interacting with the communication network of other competing microorganisms. For the purpose of intercellular communication, microorganisms use a wide range of signalling molecules that have been called autoinducers (Engebrecht and Silverman, 1984). It is also becoming apparent that in addition to these chemical mediators, an alternative type of communication probably exists. This paper presents evidence that light emission produced by microorganisms is used as their special "language" or at least as a "dialect", suggesting a much more complex form of communication between microbes than previously thought.

The purpose of this review is to present different examples of light-mediated communication between microorganisms. But, at first, it is necessary to focus attention on the processes of light formation and its absorption.

The possible sources and targets of light

Although it is an established fact that microorganisms do produce light, no agreement has been achieved in the area of interpretation of its origin.

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According the generally accepted point of view, light emission is due to heterogeneous, localized phenomena in various compartments of the cell with different sources of emission from unrelated processes. Namely, light emission from microorganisms was experimentally attributed to oxidative side reactions accompanying protein synthesis for ultraviolet light (Konev, 1967) and to superoxide dependent lipid peroxidation for the visible light emission (Quickenden and Tilbury 1983, 1991; Cadenas and Sies, 1984; Halliwell and Gutteridge, 1989) and infrared light emission (Günther, 1990).

Alternatively, the competing standpoint maintains that cellular DNA is a high energy, electronically excited molecular complex that emits light from the UV to infrared parts of the spectrum (Nagl and Popp, 1983). In this model, light emission from DNA is energy from the cell that contains information about the state of the whole cell. The main distinguishing feature of such a kind light emission is believed to be its coherence (Popp et al., 1994). In other words, living cells are considered as a natural lasers that are polychromatic and of low intensity. Moreover, it was experimentally found that not only a single cell might be a light emitter but a community of different cells or organisms as well (Chang et al., 1998).

Although direct evidence for the biophysical interpretation of the phenomenon of light emission is still lacking, indirect evidence comes from a large number of observations. For example, the intensity of the biological light emission may be significantly increased for a small rise in toxicant concentration, contrary to standard chemiluminescence theory that predicts a linear relationship between them. Furthermore, Popp and co-workers experimentally found that elderberry leaves emit a highly coherent light, and the shape of the signal rules out the origin of the light being from chemiluminescence, bioluminescence, flourescence and super fluorescence (Popp et al., 2002).

If all microorganisms do emit the light, then how do they absorb the photons? While in eukaryotes mitochondrial cytochrome *c* oxidase is considered as a main photoacceptor molecule (Karu, 1989, 1999), in prokaryotes, it is not exactly clear what kind of molecules may serve as photoacceptors. In 1995, Afanasyeva and co-workers proposed that cytochrome *bd* and *bo* complexes might be the main photoacceptors in *E. coli* cells (Afanasyeva et al., 1995). However, it was found also that cellular DNA (the experiments were performed with *E. coli* DNA) might be involved in light absorption, especially in the indigo-blue-green region of the visible spectrum (Lage et al., 2000). This finding supports the prediction of Popp and others that light might be trapped and re-emitted by DNA. In summary, in microorganisms the photoacceptor molecules and the primary mechanisms of light action have not yet been established, and further research is needed to reveal them.

The historical data and current research

Investigations in the area of light-mediated communication among microorganisms has a long history. Namely, it started immediately after the discovery of mitogenetic radiation (MR) by Alexander Gurvitch in the 1920s (Gurvitch, 1926). He discovered that onions kept closely together stimulate growth of each other's roots. The author separated the roots by encasing them in different materials and showed that this was not simply a chemical influence. One important finding Gurvitch made was that the effects of growth stimulation occurred when quartz was used but not UV-opaque glass. Thus, he hypothesised that radiation, emitted by one onion and absorbed by another, belonged to in the ultraviolet spectrum of light and takes part in the transfer of some information concerning the rate of cell division. A short time later, different species of microorganisms were involved in the research, including *Bacillus mesentericus* and *B. lactis aerogenes* (Sewertzowa, 1929), *B. murimoris* (Acs, 1931), and *Staphylococcus aureus* (Wolff and Ras, 1931). All these studies on the ability of information exchange by means of electromagnetic fields between microorganisms have been summarised in the book of Rahn (1936). Unfortunately, the experimental design of these early works did not exclude the possibility of metabolite exchange between the cultures under study. Therefore, these early works will not be discussed in this mini-review.

The interest in the problem has been rekindled in the beginning of the last decade. The first work in this area was by Nikolaev (1992). A special "flask in a flask" device was used for bacterial cultivation (Fig. 1). The inner flask was made of quartz glass and its neck was outside of the device. Two cultures of *Vibrio costicola* referred to as emitter and recipient were cultivated in the big outer flask and in the smaller inner flask, respectively. Liquid nutrient media (beef-extract broth supplemented with inorganic salts) was used for culture growth in the both flasks. Growth was monitored using light scattering by measuring the OD_{540} value, which was measured in quartz cuvettes and a 5-mm light path with the use of a KFK-2 spectrophotometer. In the control experiments, the recipient culture was

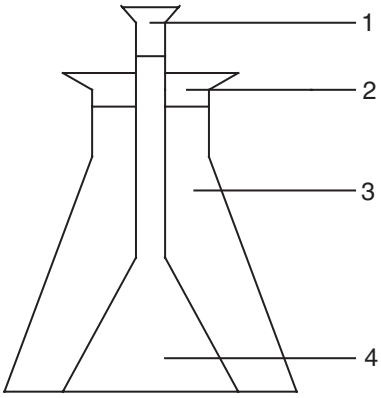

Figure 1. Experimental set-up "flask in a flask" for investigation of communication between not-physically asssociated bacterial cells (cited from Nikolaev, 1992): (1) screw-cap of inner flask; (2) screw-cap of outer flask; (3) outer flask with emitting culture; and (4) inner flask with receiving culture.

grown without the emitter culture: there was water in the outer flask. It was found that a chloramphenicol-treated (the final concentration of the antibiotic was 100 μg/ml) culture of *V. costicola* (signal emitter) could stimulate the growth of a recipient culture of the same species. The biomass increase of recipient culture was not substantial (mean 6.4 ± 2%), but the author claimed that the results were statistically significant. It is important to note that there was no influence of a non-chloramphenicol treated emitter culture on the growth of the recipient culture. Furthermore, there are other serious objections to Nikolaev's experiment. First, it is unclear in the paper why the indicated concentration of the chloramphenicol was used? Was the level of chloramphenicol sufficient to completely block translation and hence growth of the emitter? It would be well to study the plating efficiency of the chloramphenicol-treated emitter culture. Furthermore, the picture would be clearer if the author investigated the interaction of both emitter and recipient cultures that were treated with antibiotic. Second, the use of water in the control experiments was not legitimate because, as it has been shown previously, sterile growth medium might be a source of light emission due to the Maillard reaction (Wondrak et al., 1995; Vogel et al., 1998). Thus, it would be better to use the same growth medium in the control experiments. Third, it is distinctive that the effect observed was very small and the number of experimental repetitions (2–4) was insufficient to affirm with confidence the culture-to-culture influence. Nevertheless, even though the effect was claimed by Nikolaev to be statistically significant, these other objections raise concerns about the possible mechanisms of the phenomenon observed.

Nikolaev postulated a light-mediated interaction between the bacterial cultures under study. However, other possible explanations of the phenomenon must be eliminated before the presentation of such a conclusion. Also, the author did not explain how the samples for measuring the OD value were picked out. This is an important detail since metabolite exchange might be possible during OD measurements. In this respect, additional questions are raised: was the chemical disconnection between the emitter and recipient total? Was the quilted tap sufficient to prevent the transmittance of chemicals between the cultures under study? Moreover, the device used was enveloped in aluminium foil and hence it might cause the increase of concentration of volatile metabolites if an imperfect chemical separation existed. Therefore, the interaction between emitter and recipient culture might be caused by chemicals such as the volatile signals of *Ralstonia solanacearum*, which is active in 10^{-9} M range (Flavier et al., 1997). Moreover, if the microbial interaction observed was mediated via an electromagnetic field, as the author proposed, in this experimental scheme it is impossible to separate the precise mode of the signals—was it a sonic or a light signal? The question remains unsolved.

In 1997, work was published in which the example of communication by means of light between the bacterium *Pseudomonas corrugata* and the fungus *Gaeumannomyces graminis* var. *tritici* was considered (Wainwright et al., 1997). The authors used a device similar to the "flask in a flask" device: an outer cylindrical vial, closed by a glass lid, containing an inner one. The walls of the inner and outer vials were separated by small glass protrusions. The outer cell and lid were always made of either UV-opaque or UV-transparent glass. The outer vial with nutrient medium was inoculated with *G. graminis* (signal sender), the inner vial contained a culture of *P. corrugata* (signal receiver). Bactreial culture of *P. corrugata* was marked with *luxAB* genes, which are responsible for light emission. Wainwright and co-workers detected stimulation of bacterial growth in the presence of a growing fungal culture. The increased bacterial growth was monitored with the use of LKB 1251 luminometer. This effect was not observed if the authors used an UV-opaque inner flask. This suggests that UV light served as the signal and that UV light or another stress is required for the signal to take effect.

However, this study again raises many questions. The authors have not, nor attempted to, explain

why were these strains of microorganisms were chosen? Is the interaction between *G. graminis* and *P. corrugata* possible in nature? If so, then it was probably not necessary to change the wild genotype of *P. corrugata* by introducing foreign *lux AB* genes into the bacterium. Regards to growth monitoring of signal receiver bacteria, it would be better to conduct a parallel measurements of optical density since there are a lot of facts for non-linear dependence between the level of light emission and number of cells (Chang et al., 1998). Concerning any chemical interactions between the microorganisms under study, the authors emphasized that this was a possibility. When the authors tried to cultivate the signal receiver (bacterial cells) in the sealed vial, the effect was absent. Wainwright and co-workers explained this fact by the lack of oxygen that is indispensable to bacterial growth and metabolism. However, it is impossible to clearly detect the reason for the diminution of bacterial light emission. Was it really due to lack of oxygen or because of interruption of chemical signalling between the fungus and bacteria? At the same time, it is not clear why the effect of microorganism interactions occurred only when the UV-transparent inner flask was used (on the assumption that chemical signalling is possible in both cases of UV-opaque and UV-transparent inner flasks utilization). Was it the synergistic effect of both chemical and UV-light signals? Despite these unanswered questions, it is important to note that the absence of the effect during bacterial cultivation in UV-opaque and the sealed inner flask eliminates the possibility of a sonic mode of communication between *G. graminis* and *P. corrugata*. Work on possible sonic communication in bacteria had previously been published (Matsuhashi et al., 1995) and it is unfortunate that the authors did not given more attention to this question. Wainwright wrote that the effect observed varied significantly (from a few percent to a 30-fold increase), and it was not always reproducible (it occurred only in two of seven experimental runs). Unfortunately, he and his co-authors did not explain the inability to repeat some of these experiments. Evidently these effects appeared to depend significantly on several parameters both physiological and physical. In similar experiments on light-mediated communication in cultured human tissues, Kaznacheev and Mikhailova (1981) showed that the effects of culture-to-culture interaction depended on external influences such as season, sun activity, etc. Unfortunately, after 1997 there were no new publications of Wainwright and co-workers dedicated to the problem.

In 2000, Nikolaev reported about the communication by means of light in *Pseudomonas fluorescens* (Nikolaev, 2000a). The author used the ''flask in a flask'' device but the inner compartment was made from usual glass instead of quartz. Sender and receiver cultures were cultivated in a liquid nutrient medium M9 supplemented with glucose and mineral elements in the outer and inner flasks, respectively. The outer flask and the inner one were separated with the use of rubber membrane. Sender and receiver cultures had different initial optical densities (OD_{600}): 0.05–0.1 and 0.6–0.8, respectively. OD values were measured with the use of Pye-Unicam SP-450 spectrophotometer. The effect of interaction of bacterial cultures with one another was studied by determining the number of cells adhered to glass and the number of non-adhered free cells. To estimate the value of adhesion (% from initial number of cells), the following equation (Nikolaev, 2000b) was applied: $(OD_{initial}-OD_{minimal}/OD_{initial})$, where $OD_{initial}$ and $OD_{minimal}$ are optical densities at the moments of inoculation and maximal reduction of OD after inoculation, respectively. The number of free cells was calculated as follows: $OD_{min\ exp}/OD_{min\ con}$, where $OD_{min\ exp}$ and $OD_{min\ con}$ reflects the number of cells in the presence of a sender culture and without a sender culture, respectively. Nikolaev found a 50% increase in the number of free (non-adhesive) cells, and the value of adhesion was decreased by 4% (Nikolaev, 2000a). It should be noted that in the scheme of the experiment gaseous exchange between sender and receiver was eliminated.

In another study Nikolaev (2000b) showed that there was a special chemical (he called it ''volatile anti-adhesin'', VAA), that was responsible for the decrease in cell adhesion (it caused a 6% diminution in cell adhesion). The author decided to investigate the character of the distant interaction between sender and receiver cultures of *P. fluorescens* investigating both of the factors above (chemical and electromagnetic). Nikolaev observed a significant reduction in the number of non-adhered cells (mean 9-fold) due to chemical and electromagnetic interaction between the sender and receiver cultures. He concluded that there was a synergistic effect between electromagnetic and chemical signals.

Concerning the work discussed above, a few notes should be taken. The discovery of synergistic effect of both chemical and electromagnetic signals action is highly attractive. But a question arises from the finding: does the chemical signal (VAA) modulate the action of the electromagnetic signal or vice versa? Regarding the electromagnetic

nature of a signal, the author considered that it was not UV light, since the flasks employed were made from ordinary glass, which is non-transparent for the corresponding spectral range. The author did not point out the wavelength of visible (and/or infrared?) light, which was responsible for the effect observed. Moreover, the experimental design of this work did not exclude a possible sonic signal since there were no control experiments, in which the interacting cultures were isolated optically. And what about VAA? What is the precise chemical nature of the volatile substance?

Lately, I have investigated the distant regulation of bacterial growth and light emission of mechanically and chemically separated bacterial cultures (Trushin, 2003a). The experiments were performed with *Escherichia coli* cells cultivated in a specially constructed device, which was made from UV-opaque glass. There are two equal compartments separated by a window made from UV-opaque glass (Fig. 2). Different nutrient media were used for culture growth (LB and M9 supplemented with glucose). Bacterial cultivation was performed in the dark and growth was monitored with the use of Specord M40 spectrophotometer. Furthermore, light emission from cultures within both compartments of the device was measured.

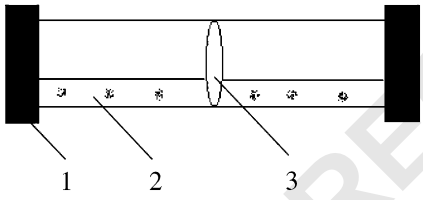

Figure 2. Experimental set-up for investigation of optic interactions between bacterial cells (cited from Trushin, 2003a): (1) screw-cap of cylinder; (2) medium with growing cells; and (3) glass window (opaque and clear).

I found that the values for the duration of the lag phase of bacteria grown in M9 medium were greater than those of the control. There were no statistically significant differences in the duration of lag phase during LB cultivation. In both media used, the values for the growth rate of the cultures cultivated jointly in the device were greater than the control ones. Concerning the harvest, there was no statistically significant difference between the cultures under study and the controls during M9 cultivation. When the cultures were grown in LB medium, the harvest values were less than the control ones. It is essential to indicate that a link between light emission and the growth parameters was observed. The changes in the growth of cultures under study correlated with modifications in light emission. The most interesting finding is the phenomenon of synchronization in the emission spectra: impressive results were obtained during the phase of active growth in both media used (Fig. 3). It is noted that bacteria in the joint compartments of the device used have not been synchronized by use of specific methods (for example, by the method of amino acid starvation). Probably, the synchronization in growth and light emission occurred due to electromagnetic link between the separated cultures. Thus, the alteration of bacterial growth and the synchronization of light emission of interactive cultures were the main observations of my research supporting the statement that the cultures of *E. coli* are able to interact at a distance via electromagnetic fields.

Inasmuch as in the experiments above the joint cultures were grown in equal conditions (i.e., without any additional influence on any of them), an investigation of optical interaction of bacterial cultures in the case when one of them was impacted with some damaging or stimulating factor was of big interest. With this aim, one of the cultures was irradiated with both red and infrared light (Trushin, 2003b). Other experimental tasks

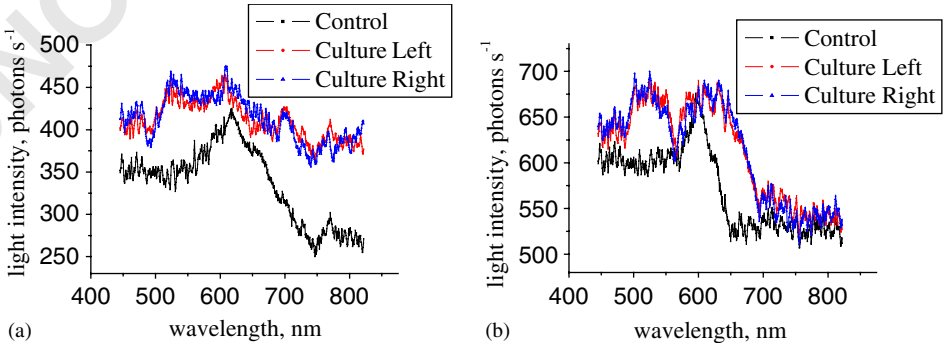

Figure 3. The radiation spectra of the *E. coli* MC1061 during exponential phase of growth: (A) cultivation in M9 medium; and (B) cultivation in LB medium (cited from Trushin, 2003a).

were: (i) to study the red and infrared light effects on the *E. coli* growth rate under conditions of optical interactions from irradiated and non-irradiated bacterial cultures; (ii) investigation of the character of optical interaction between irradiated and non-irradiated cultures. *E. coli* cells were irradiated with red and infrared light at a dose of 6 kJ/m^2 and cultivated in conditions that were identical to those previously described (Trushin, 2003a). The main finding was a reduction of the growth-stimulating effect of red and infrared light when optical interaction occurred between an irradiated culture and non-irradiated one. The extent of reciprocal growth stimulation was also less but only during M9 cultivation. On the contrary, there was a significant mutual growth enhancement when the cultures were grown in LB medium. The possible explanations of the phenomenon are discussed (Trushin, 2003b).

As regards the mechanisms of the phenomena above, one of my conclusions is that the results obtained cannot be explained by the cultures interacting in the UV range of the spectrum. The devices used to culture the bacteria were made from glass, which absorbs a bigger part of UV radiation. Furthermore, a sonic nature of the interaction must be excluded because there were no statistically significant effects during cultivation of cultures in the device with an opaque glass window between the adjacent compartments in both of aforementioned experiments. Thus, I concluded that the most appropriate candidates for the signal are visible light or IR. However, this should be clarified in future studies. And finally, the chemical communication in my experiments was totally excluded since the samples were taken with the use of sterile syringe thorough a rubber septum. In this scheme of sampling, release of volatiles was less probable.

Not long ago, some new data concerning microbial communication were published. In Heal and Parsons' article (Heal and Parsons, 2002), antibiotic resistance due to culture-to-culture interaction was investigated. The authors examined the ability of one *E. coli* 24 h-culture to strengthen the growth of another culture of the same species under antibiotic stress. Heal and Parsons found that the signal receiving population, only in the neighbourhood of the signal transmitting one, was able to grow on ampicillin-containing (500 ng/ml) solidified LB medium in a bi-partite Petri dish (Heal and Parsons, 2002). There was no effect of antibiotic resistance when the air gap between the compartments with signal transmitting and signal receiving bacterial populations was plugged. Although Heal and Parsons claimed that Parafilm was used to prevent an air passage between the cultures, it is not clear how this was done. Moreover, the effects described by Heal and Parsons were dependent on the distance between the populations and it was significantly decreased at distances greater that 3 cm (Heal and Parsons, 2002). Since indole has been shown to be released during the exponential phase of growth and could regulate the expression of amino acid metabolism genes, the authors proposed that it is responsible for the phenomenon. However, stronger evidence to substantiate this assumption is still lacking. It is also necessary to note that in this experiment, as with the others, there was a large variation in the level of the effect. This could be linked to a synergistic volatile chemical and physical field effect. Unfortunately, this paper did not consider this. In this connection, it would be interesting to study whether antibiotic resistance is conferred when the Petri dishes with signalling and receiving populations are stacked on top of each other.

Since 1994, an interesting series of articles dedicated to cell-density dependent effects of extremely high- and low-frequency electromagnetic fields as well as low dose ionizing radiation on bacterial cells were published (Belyaev et al., 1994, 1995, 1998, 2000; Shcheglov et al., 2002; Alipov et al., 2003). To investigate the changes in the genome conformational state induced by extremely low-frequency electromagnetic field (ELF EMF) in *E. coli* cells, the method of anomalous viscosity time dependence (AVTD) was used (Belyaev et al., 1995). Belyaev and co-workers found that the effect of ELF EMF depends on the cell concentration in the bacterial culture and it was maximal at a concentration of about 6×10^8 cells/ml (Belyaev et al., 1995). The authors suggested that cells were able to interact under the influence of ELF EMF, and possible explanations of the phenomenon were made. The obtained data, including kinetics and cell-density dependencies for the observed effects fitted better to an electromagnetic mechanism. However, chemical compounds with a short life span could also account for the observed effects (Belyaev et al., 1995).

The reaction of *E. coli* cells to microwaves of extremely high-frequency range (millimeter waves, MMW) with different power output also have been studied (Belyaev et al., 1994, 2000; Shcheglov et al., 2002). The same method, AVTD, was applied to the investigation of MMW effects on microorganisms. As before, the cellular cooperativity in response to MMW was observed and the bacterial response to microwaves were altered depending on the stage of growth (Belyaev et al., 1994, 2000; Shcheglov et al., 2002). During logarithmic growth,

there was a reduction in the value of AVTD due to MMW exposure, with a maximal effect observed at 10^{-3} W/cm^2 power output (Shcheglov et al., 2002). At the stationary phase of growth, microwaves exposure resulted in an increase in AVTD at both power outputs (10^{-18} and 10^{-3} W/cm^2) (Shcheglov et al., 2002). In such a way, cooperative cellular responses to both ELF EMF and MMW was found. The maximal effect resulting in alterations of AVTD values corresponding to stationary phase cells (Belyaev et al., 1994, 1995, 1998, 2000; Shcheglov et al., 2002).

According to the theoretical model proposed (Belyaev et al., 1995), the communication of bacteria could be mediated by emission of electromagnetic waves in the infrared-sub-millimeter range (Belyaev et al., 1995, 1998; Shcheglov et al., 2002). This conclusion was in agreement of Frohlich's prediction about coherent excitation in biosystems (Frohlich, 1968). The main supporting evidence for this hypothesis was the fact that the effect of bacterial cooperativity was found at the cellular density of about $(4–6) \times 10^8$ cell/ml. The intercellular distance at this cellular density is about 30 μm, which corresponds to absorption length of the aforementioned electromagnetic spectrum. It is necessary to note that the Belyaev and co-workers tried to test the possible chemical nature of the bacterial cooperative response to ELF EMF (Belyaev et al., 1998). With this aim, the exposed cultures were filtrated or spun down and the obtained media was added to the unexposed cells. There were no changes in AVTD parameters in this case or after ELF EMF-treated growth medium (with possible chemicals released by bacteria but without cells) was added to another bacterial culture before its exposure (Belyaev et al., 1998). Although Belyaev and Scheglov with co-workers did not totally exclude a possible chemical mechanism for the effects observed, they consider that the electromagnetic hypothesis is a more suitable explanation of the type of cellular communication observed (Belyaev et al., 1995, 1998; Shcheglov et al., 2002).

Similar effects of intercellular communication were observed in response to ionizing radiation (Alipov et al., 2003). *E. coli* cells were treated with ionizing radiation in the range of 0.1 cGy–1 Gy , and cellular lysates were assayed for genome conformation state with the use of AVTD method. Alipov and co-workers found that the values of relative viscosity were greater at a greater cell density (4×10^{-8} cell/ml in comparison to 4×10^{-7} cell/ml). So, the character of cellular cooperative response was similar to those for ELF EMF and MMW range. Therefore, it was suggested that the mechanism above, which was developed for ELF EMF and MMW, is also suitable for explanation of intercellular communication during X-ray exposure (Alipov et al., 2003). The analogous cooperative cellular response to ionizing radiation was found in mammalian cells, and it was regarded as a "bystander effect" (Azzam et al., 1998; Mothersill and Seymour, 2000; Zhou et al., 2000; Belyakov et al., 2001; Sawant et al., 2001; Ward, 2002; Ŏsterreicher et al., 2003). Although little is known about the precise mechanisms of bystander effects, it is reasonable to propose that the phenomenon is rather universal and its mechanisms are similar to those for bacteria.

The common drawbacks of the aforementioned experiments

Although I have made some very critical remarks regarding the above-discussed data, there are several general themes that come from this critique. In this section, these common shortcomings will be considered.

First, none of the authors have taken into serious account the physical properties of the glass devices used. Instead of this, it was postulated that the devices used were made of quartz (Nikolaev, 1992; Wainwright et al., 1997) or "usual" glass (Nikolaev, 2000a, b; Trushin, 2003a, b). Except for my experiments, it was not stated exactly what type of quartz or "usual" glass was utilized for the production of the devices used. Needless to say that the chemical composition of any glass determines significantly its features. For example, fused silica and crystalline quartz differ in the content of inorganic elements and vary also in transmittance range (150–3000 nm for fused silica, and 200–2000 nm for crystalline quartz) (Holloway, 1973). So, in the experiments where UV light was stated as an informational signal (Nikolaev, 1992; Wainwright et al., 1997), it is really unclear what exact wavelength was responsible for the effects observed. Except for my own experiments, the type of "usual" glass was not also indicated. It means that it is impossible to define whether visible or infrared signals are involved since the transmittance range also varies among "usual" glasses. Unfortunately, the electrical and thermal properties of glass were not also considered.

Second, there was no stringent control for diffusion of signalling volatiles in experiments on light-mediated bacterial communicaton. In this respect, the use of radioactive substances (for example, ^{3}H-leucine) would be helpful to eliminate

the possibility of chemical transfer during the experiments. It is possible that microdoses of some volatiles caused the macroeffects observed.

Third, it is unclear in the experimental design whether the microorganisms were synchronized or not. Did they grow in the dark or not? Except for my own experiments, microorganisms under study were not optically isolated. The simple optic isolation might be useful for decision making about whether the interactions observed were truly light mediated. Similarly, the obtaining of light emission spectra is necessary for making conclusion with regards to possible mechanisms of the phenomena.

Another criticism concerns statistical treatment of the data obtained. In the aforesaid experiments, different statistical criteria were used with both parametric and non-parametric tests. Namely, Nikolaev's experiments were performed in 6–12 replicates (1992) and 10–15 replicates (2000), and he used tests of Student's *t*-test and Wilcoxon (1992, 2000). Wainwright's studies were done in triplicate and Student's *t*-test was used (Wainwright et al., 1997). In my own experiment, I used the tests of Kolmogorov–Smirnov (2003a), Shapiro–Wilk (2003b) and Student's *t*-test (2003a, b), and experiments were done in 14 (2003a) and 10 (2003b) replicates. Regards to Nikolaev's experiments, it is not entirely clear why both the parametric and non-parametric tests were used. He did not define in his papers whether the testing of normality was done. If it was done, then the use of Student's *t*-test was legitimate, and the utilization of Wilcoxon test was not necessary. In Wainwright's studies, the testing for goodnes of fit to a normal distribution was also not performed. In my experiments, I tried to escape these shortcomings. Therefore, before the use of a Student's *t*-test, the experimental data were analysed for goodnes of fit to normal distribution using the tests of Kolmogorov–Smirnov (Trushin, 2003a) and Shapiro–Wilk (Trushin, 2003b). As for further research in this controversial field of microbiology, one must keep in mind that a more attention should be given to statistical treatment of experimental data.

Up to now, in the scientific literature there is no data in support of results that have been presented in this paper. This situation might be explained in a few ways. First, none of the researchers have been successful in performing analogous studies. This might be due to non-observance of experimental design or due to experimenter bias. In this connection, the use of double-blind protocol, as it was done by Wainwright and co-workers (Wainwright et al., 1997), would be helpful. Second, there were some successful attempts in repetition of such a kind experiments but they passed over it in silence since the publishing repeat experiments is more difficult to achieve.

Conclusion

A few decades ago, little was known about the ability of microorganisms to coordinate behavior in a cell-density-dependent fashion. However, much of the progress in the area of chemical communication in microorganisms has been achieved due to a revolution in molecular biology that has greatly extended the range of techniques available for research of microbial genetic and biochemical systems. Now, one can see that our knowledge of how bacteria communicate with each other via different signalling compounds has been elucidated. Along with it, we now know that not only chemical communication but also a light-mediated communication appears to exist in microorganisms. Most likely living organisms have evolved complementary types of light-mediated and chemical communication.

Despite the numerous drawbacks in the experimental design, the phenomenon of light-mediated communication seems to be legitimate. Besides microorganisms, similar results were obtained in experiments with seedlings of garden radish and barley (Kuzin, 2002), pollen of cherry and plum (Budagovskii et al., 2001), rat tumor cells (Kirkin, 1981), amniotic and nephritic human cultures (Kaznacheev and Mikhailova, 1981), BHK cells (Albrecht-Buehler, 1992), fish eggs, embryos and larvae (Beloussov et al., 2002), beetles and daphnia (Chang et al., 1998). Moreover, it was found that not only light but also sound appears to be involved in the regulation of different processes in bacteria (Matsuhashi et al., 1995, 1996, 1998). Apparently, the list of living organisms that utilize different physical signals for communication will grow.

We are now probably at the beginning of a new era, with advanced technologies giving more opportunities to rapidly enhance our understanding of various communication systems in microorganisms. It is my deep conviction that the scientific community should focus efforts on investigation of physically mediated communication instead of simply rejecting this possibility to communicate. In order to shed light on the mechanisms of this mode of communication, biologists, physicists, physicians and other specialists should be involved in this intriguing study.

Uncited references

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