



LIPPINCOTT
WILLIAMS & WILKINS

C-ode: 3642

To: Dr. Maxim V Trushin
Fax: 7-8432-387577
Date: September 9, 2003

From: Emily Tsui
Fax: (852) 2421 1123
Total: 10 pages (include cover page)

Dear Dr Trushin

Journal of Microbiology, Immunology and Infection and Reprint Order Form

Enclosed herewith your article titled "The possible role of electromagnetic fields in bacterial communication" for your proofreading.

Please note:

1. Your article has been edited by the Editorial Board of the Journal and by us. Under the principle of consistency, we unify writers' writing styles such as the use of numbers, hyphenations, compound words, reference style. You may ignore this part while proofreading.
2. After your checking, the proofs would be sent to Journal's Chief Editor for final proofreading before printing.
3. Please mark your corrections neatly and clearly on the proofs. Due to the limited time, please fax to us your corrected proofs **BEFORE September 11, 2003. Any corrections made after this deadline will not be processed.**
4. Enclosed please find a Reprint Order Form. Please kindly circulate to other authors of the article for their ordering.

✠ **As a confirmation that the article has been proofed, please kindly sign on the first page of the article and fax me back. Please do so even if you have made no amendments.**

Your kind cooperation is very much appreciated. Thank you for your invaluable contribution to the journal.

Yours sincerely

Emily Tsui



Lippincott Williams & Wilkins Asia Limited

Suite 907-910, Wharf T & T Centre, Harbour City, 7 Canton Road, Tsim Sha Tsui, Kowloon, Hong Kong
Tel: (852) 2610 2339 • Fax: (852) 2421 1123 • E-mail: weserve@lww.com.hk • LWWW.com

The possible role of electromagnetic fields in bacterial communication

Maxim V Trushin

Kazan Institute of Biochemistry and Biophysics, Kazan, Russia

Received: Accepted:

This paper presents a general review of the current knowledge regarding bacterial communication via electromagnetic fields. The possible role of ultraviolet, visible and infrared light, extremely high frequency, and low frequency electromagnetic fields as well as sound waves is discussed. The probable mechanisms of remote microbial interactions are rising. Future trends in the area of microbiology are also viewed.

Key words: Bacterial communication, electromagnetic fields

For a long time, the ability to communicate has been considered as an exclusive property of multicellular organisms. However, research over the past few decades has raised more than serious doubts about this point of view. Complex behaviors such as chemotaxis, quorum sensing, and biofilm formation show that bacteria can communicate with the environment, within the species, and with other species with the use of chemical signaling molecules (pheromones) [1-3]. The chemical mode of communication is the best studied of all. Nevertheless, bacteria also use electromagnetic signals as part of sophisticated signaling system that function over distances that are substantially larger than cellular dimensions (which are the order of one to a few micrometers). The following descriptions focus mainly on the investigations in the area of electromagnetically mediated communication of microorganisms.

Research into the electromagnetically mediated communication of microbes started immediately after the discovery of mitogenetic radiation (MR) by Alexander Gurvitch in the 1920s [4]. His observation stimulated early research, which led to over 500 publications on the ability of information exchange by means of electromagnetic fields between microorganisms [5]. However, these early works will not be discussed in this review.

Today, there are 2 basic explanations of the nature of electromagnetic communication in microorganisms. Some authors suggest that effects observed are connected with the transmission of light signals, others

insist on the sonic nature of the mode of bacterial communication.

Light-mediated Communication

In the early 1990s, the first work in this area was by Nikolaev [6]. A special device "flask-in-flask" was used for bacterial cultivation. 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 a big outer flask and in a smaller inner flask, respectively. Liquid nutrient media were used for culture growth in the both flasks. Growth was monitored using light scattering by measuring the OD₅₄₀ 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 grown without emitter one: there was water in the outer flask. It was found that the chloramphenicol-treated (the final concentration of the antibiotic was 100 µg/mL) culture of *V. costicola* (signal emitter) could stimulate the growth of 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 know that there was not any influence of non-treated with chloramphenicol emitter on the growth of recipient culture.

However, there are some 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 of the developments

Corresponding author: Dr. Maxim V Trushin, Kazan Institute of Biochemistry and Biophysics, Lobachevskiy Street 2/31, P.O. Box 30, 420111, Kazan, Russia. E-mail: mirushin@mail.ru

observed would be clearer if the author investigated the interaction of both emitter and recipient treated with antibiotic. Second, it is distinctive that the effect observed was too small and the number of experimental repetitions [2-4] was insufficient to affirm the culture-to-culture influence. Nevertheless, if the effect was really statistically significant as Nikolaev claimed in his paper, the other objections should be raised concerning the possible mechanisms of the phenomenon observed.

The author did not work out in detail the nature of the signal. He only postulated the electromagnetically-mediated interaction of the bacterial cultures under study. However, the 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 connection, 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 aluminum foil and hence it might cause the increase of concentration of volatile metabolites in the case of imperfect chemical cultures separation and the interaction between emitter and recipient culture might be caused by some chemicals like volatile signals of *Ralstonia solanacearum*, which is active in 10-9 M range [7].

Finally, if the microbial interaction observed was mediated via electromagnetic field, as the author proposed, in this experiment it is impossible to separate the precise mode of the signals -- was it sonic or light signals? The question remains unsolved.

Another work also demonstrates the example of communication by means of light between the bacterium *Pseudomonas corrugata* and the fungus *Gaeumannomyces graminis* var. *tritici* [8]. The authors used the device like "flask-in-flask": the outer cylindrical vial, closed by a glass lid, contained 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). Wainwright and coworkers detected stimulation of bacterial growth (that was observed from light emission) in the presence of a growing fungal culture. This effect fails if the authors used 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. The effect observed varied significantly (from a few percents to a 30-fold increase), and it was not always reproducible (it occurred only in 2 of 7 experimental runs). Despite this, the authors insist that they revealed a electromagnetic link between bacterial and fungal cultures.

Like any research, this study raises many questions. The authors have not, nor attempted to, explain why these strains of microorganisms were chosen. Is the interaction between *G. graminis* and *P. corrugata* possible in wild nature? If yes, then probably it was not necessary to change wild genotype of *P. corrugata* introducing a foreign *lux AB* gene into the bacterium. Concerning the chemical interactions between the microorganisms under study, the authors emphasized that the possibility was real. When the authors tried to cultivate the signal receiver (bacterial cells) in the sealed vial, the effect was absent. Wainwright and coworkers explained this fact by the lack of oxygen that is indispensable to bacterial growth and metabolism. However, in this condition, 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 signaling between the fungus and bacteria? At the same time, it is not clear why the effect of microorganism interactions occurred only when UV-transparent inner flask was used (on the assumption that chemical signaling 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 signal action? Despite the unintelligible explanation from the direction of the authors on the above questions, it is important to note that the absence of the effect during bacterial cultivation in UV-opaque and sealed inner flask eliminates the possibility of sonic mode of communication between *G. graminis* and *P. corrugata*. By that time, work on the sonic communication in bacteria already had been published [9] and the authors should have given more attention to this question. Unfortunately, after 1997 there were no any new publications of Wainwright and coworkers dedicated to the problem.

More recently, a paper was published in which the communication by means of electromagnetic fields in *Pseudomonas fluorescens* was described [11]. The author used the "flask-in-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 outer and inner flasks, re-

spectively. 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): 0.05 to 0.1 and 0.6 to 0.8, respectively. The effect of interaction of bacterial cultures with one another was studied by determining the number of adhered and non-adhered free cells. To estimate the value of adhesion (% from initial number of cells), the following equation [11] was applied:

$$OD_{\text{initial}} - OD_{\text{minimal}}/OD_{\text{initial}}$$

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:

$$OD_{\text{min exp}}/OD_{\text{min con}}$$

Where $OD_{\text{min exp}}$ and $OD_{\text{min con}}$ reflects the number of cells in condition of distant interaction of sender and receiver and without the interaction, respectively. Nikolaev found an increase in the number of free (non-adhesive) cells by 50%, a decrease in the value of adhesion by 4% [10]. It should be noted that in the scheme of experiment the gaseous exchange between sender and receiver was eliminated.

Since in his own previous work [11], Nikolaev showed that there is a special chemical (he called it "volatile anti-adhesin"; VAA) that is responsible for the decrease in cell adhesion (it caused a 6% diminution in cell adhesion), the author decided to investigate the character of distant interaction between sender and receiver cultures of *P. fluorescens* in the case of action of both the factors above (chemical and electromagnetic as well). 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. Nikolaev concluded that there was a synergistic effect between electromagnetic and chemical signals.

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 one 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. And what about VAA? What is the precise chemical nature of the volatile substance? Turning back to the electromagnetic signal,

the author did not point out, for example, the wavelength of visible (and/or infrared?) light, which is responsible for the effect observed. Moreover, the experimental design of this work did not exclude the sonic nature of the signal. Despite this, Nikolaev did not discuss the possibility.

In my recent work, I investigated the distant regulation of bacterial growth and light emission of electromagnetically separated bacterial cultures [12]. The experiments were performed with *Escherichia coli* cells cultivated in a specially constructed device, which was made from UV-opaque glass. There are 2 equal compartments separated with the window from UV-opaque glass. Different nutrient media were used for culture growth (LB and M9 supplemented with glucose). Growth was monitored with the use of Specord M40 spectrophotometer. Furthermore, a light emission from cultures of both compartments of the device was measured.

This work 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 cultures under study and the control ones during M9 cultivation. When 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 growth of cultures under study have correlated with modifications in light emission. The most interesting finding is the phenomenon of synchronization in the emission spectra. It is noted that bacteria in the joint compartments of the device used have not been synchronized by use of a specific methods (for example, by the method of amino acid starvation). The synchronization in growth and light emission have probably 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.

Since in the experiments above the joint cultures were grown in equal conditions (ie, without any additional influence on any of them), investigation of distant interaction of bacterial cultures in the case when one of them was impacted with some damaging or

stimulating factor was of a big interest. With this aim, one of the cultures was irradiated with red and infrared light [13]. Other experimental tasks were: (1) 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; and (2) investigation of character of distant interaction between irradiated and non-irradiated cultures. *E. coli* cells were irradiated with red and infrared light in dose of 6 kJ/m² and cultivated in conditions, which were identical to previously described [12]. The main finding was a reduction of growth-stimulating effect of red and infrared light in conditions of distant interaction between irradiated culture and non-irradiated one. The extent of reciprocal growth stimulation was also less but during M9 cultivation. On the contrary, there was a significant mutual growth forcing when cultures were grown in LB medium. The possible explanations of the phenomenon are discussed [13].

Concerning the mechanisms of the phenomena above, one of my conclusions is that the results obtained 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. 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 infrared. However, it 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 the rubber septum. In this scheme of sampling, release of volatiles was less probable.

Sonic-mediated Communication

In 1995, Matsuhashi and coworkers showed that one population of *Bacillus carboniphilus*, a strain isolated by them that requires carbon for growth under stress, enabled a second population of this bacterium, located in the another part of bipartite plastic or glass Petri dish, to grow under salt stress (1%-2% w/v KCl) and elevated temperature (44°C) [9]. The authors understood that there is a possibility for chemical interaction between the bacterial populations. Therefore, to prevent the gaseous exchange between sender and receiver through the air gap, the following experiments were done. One Petri dish was pre-grown with a signal sender population for 1 day. After that, a second Petri dish (salt non-

permissive conditions) was inoculated with a signal receiver cells. The 2 Petri dishes were stacked over (cover to cover). The spore germination in the receiver population after 2 days of incubation at 44°C was studied. Matsuhashi and coworkers found that the spore germination under severe conditions was more efficient when *Bacillus subtilis* was used as signaling population and *Bacillus carboniphilus* as the sensitive, signal-receiving population.

In the above work, Matsuhashi and coworkers for the first time postulated a sonic nature of the signals. However, both the sender and receiver bacterial populations were grown in the plastic Petri dishes, which are transparent, for example, for visible light. Although the specific experiments were not done (eg, envelopment of the dishes in black paper), the authors did not consider communication via light. Moreover, the fact that the interaction of sender and receiver bacterial populations was more effective when the dishes were placed into acrylic box [9] is a big suspicious. Since the authors did not specifically prevent the volatiles leak from the dishes, a high concentration of the volatile matter in the closed space of the acrylic box could have favored chemical communication between the cultures under study.

Matsuhashi and coworkers have also investigated the effects of bacteria upon drug resistance of remote neighboring bacteria [14]. Experiments were performed using Petri dishes parted into 2 equal compartments. The authors found that growing cells of *B. subtilis* (signal emitter) stimulated erythromycin and streptomycin resistance in signal recipient cells of *B. carboniphilus*. The same objections mentioned above may be applicable to the current experiment. Moreover, Matsuhashi and collaborators did not work out the hypothesis on the possible mechanisms of antibiotic resistance in signal receiver cells of *B. carboniphilus*. Even if the antibiotic resistance was occurred due to reception of sonic signals from the signal emitter cells, the most actual questions are: were the signals oriented to (1) the reduction of the uptake of erythromycin and streptomycin into *B. carboniphilus* cells, (2) alteration of bacterial targets, which became tolerant to the antibiotics; or (3) production of specific enzymes that could destroy the antibiotics or modify it, thus stimulating the drug inefficiency?

The last paper of Matsuhashi's group was published in 1998 [15]. In this work, the authors tried to replace signaling bacterial population by a sound generator. The complete set-up was composed both of a function generator (Iwatsu FG330, 600-ohm output impedance) and a speaker (Ohm SP-88 full range speaker, 8-ohm

impedance) [15]. *B. carboniphilus* cells served as a biological object, and for their cultivation, medium containing 1% w/v KCl was used. The bacterial cells under salt stress conditions were influenced by sound action from the set-up mentioned above. The effect of these sound signals was determined by efficiency of colony formation. Matsuhashi and coworkers found that there was an increased colony formation when the sound generator was switched on. Moreover, the effect depended on both audio frequencies and power output. Growth-promoting effect of sound waves was provided in the range of 10 kHz to 40 kHz at a power output of near 50 mW [15].

However, the finding that bacteria are sensitive to sound was not the direct evidence for the sonic nature of previously observed phenomena [9,14]. Therefore, in the same work the authors tried to register a sound from the bacteria. With the aim, a sensitive condenser microphone (Bruel & Kjaer, Denmark) with a preamplifier of the same manufacturing firm was used [15]. The experiments on sound registration from bacteria were performed with *B. subtilis* cells, which were grown on the medium containing polypeptone, yeast extract, glucose, sodium chloride, and thymine for maximum growth promotion. The most remarkable results was the discovery of sound emission from *B. subtilis* cells, and at the same time, the frequencies of sound registered were similar to those, which were responsible for growth promotion under stress conditions (10-40 kHz). The given finding allowed authors to propose that sound waves in the aforementioned frequency range are functioning as growth-promoting signals.

It is interesting to note that the human ear can detect sounds with frequencies at 20 Hz to 20 kHz [16]. Thus, with some license, one may say that we are partly capable to hear bacterial "conversation." Concerning the mechanisms involved in the production and reception of sound waves, Matsuhashi and coworkers made only a few assumptions. The authors proposed that different intracellular organelles, such as membranes, cytoskeleton-like structures, or the chromosome, may be responsible for sound generation [15]. Although Matsuhashi and coworkers clearly demonstrated that the signal was sound rather than light, the question of how sound is generated remains unanswered.

In 1997, an interesting interpretation of Matsuhashi's results was suggested [17]. The hypothesis of intracellular integration, which has been proposed by Norris and Hyland, is in agreement with the Frohlich prediction [18] that, under appropriate conditions,

biological objects can support a coherent excitation in the range of 10^9 to 10^{12} Hz. On basis of some literature data [19], the authors hypothesized that enzymes can be emitters of electromagnetic waves in the range above. According to this supposition, chromosomes can receive these waves, thus altering the gene activity. Regarding the results of Japanese researchers, Norris and Hyland proposed that the σ^s subunit of RNA polymerase is the most appropriate candidate for emitter of electromagnetic waves in sender bacterial population [17]. Taking into account the global regulatory role of σ^s subunit under different stresses [21,22], Norris and Hyland are inclined to believe that electromagnetic radiation from the part of RNA polymerase might promote a gene activity in receiver bacterial population [17]. Thus, a σ^s subunit of RNA polymerase, normally functioning at the stationary phase of growth, in the sender population might be responsible for growth promotion in the receiver population. In other words, as Norris and Hyland proposed, the phenomena of distant growth stimulation in Matsuhashi's experiments are consequences of enzyme activities integration in cells of both sender and receiver bacterial populations [17].

Recently, some new data concerning microbial communication were published. First of all, in Heal and Parsons' paper [23], antibiotic resistance due to culture-to-culture interaction was investigated. In this paper, the authors examined the ability of one *E. coli* culture to strengthen a growth of another culture of the same species under antibiotic stress. Heal and Parsons found that the signal receiving population, only in the neighborhood with the signal transmitting one, was able to grow on ampicillin-containing (500 ng/mL) solidified LB medium in the bipartite Petri dishes [23]. There was no effect of antibiotic resistance when the air gap between the compartments with signal transmitting and signal receiving bacterial populations was plugged. Moreover, the effects described by Heal and Parsons depended on the distance between the populations and it was significantly decreased at distances greater than 3 cm [23]. The authors proposed that indol is responsible for the phenomenon. So, the bacterial communication above was chemically mediated and, therefore, not in the interests of the current review.

An impressive series of papers dedicated to cell-density dependent effects of extremely both high frequency and low frequency electromagnetic field as well as low dose ionizing radiation on bacterial cells were published [24-29]. To investigate the changes in 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 [25]. Belyaev and coworkers found that the effect of ELF EMF depended on the cell concentration in bacterial culture and it was maximal at a concentration of about 6×10^8 cells per mL [25]. The authors suggested that cells were able to interact under the influence of ELF EMF, and the possible explanations of the phenomenon were made. Although in the experimental design of this work did exclude the chemical mechanisms of the interaction like quorum sensing in liquid medium, the authors preferred the electromagnetic mechanism of the interaction [25]. Moreover, in this work Belyaev and coworkers proposed the theoretical model for explanation of the effect of cell cooperativity [25].

The response of *E. coli* cells to microwaves of extremely high frequency range (millimeter waves, MMW) with different power output also have been studied [24,27,28]. The same method, AVTD, was applied for the investigation of microwaves effect. As before, the cellular cooperativity in response to MMW was observed and the bacterial reaction to microwaves was altered depending on the stage of growth [24,27, 28]. During logarithmic growth, there was a reduction in the value of AVTD due to MMW exposure, at that the maximal one was observed at the 10^{-3} W/cm² power output [28]. At the stationary phase of growth, microwaves exposure resulted in increase in AVTD at both power output (10^{-18} W/cm² and 10^{-3} W/cm²) [28].

Thus, cooperative cellular response to both ELF EMF and MMW was found. At the same time, the maximal effect resulting in alterations of AVTD values corresponded to stationary phase cells [24-28].

In this connection, the possible mechanisms, we have proposed to explain the phenomena observed should be considered. According to the theoretical model proposed [25], the communication of bacteria could be mediated by emission of electromagnetic waves in the infrared-sub-millimeter range [25,26,28]. This conclusion was in agreement with Frohlich's prediction about coherent excitation in biosystems [18]. The main supporting evidence for this hypothesis was the fact that effect of bacterial cooperativity was found at the cellular density about $(4-6) \times 10^8$ cell/mL. The intercellular distance at the cellular density is about 30 μ m, which corresponds to absorption length of the aforementioned electromagnetic spectrum. It is necessary to note that Belyaev and coworkers tried to test the possible chemical nature of the bacterial cooperative response to ELF EMF [26]. With this aim, the authors added the exposed cells to unexposed ones. There were no changes in AVTD parameter in this case

as well as after ELF EMF-treated bacteria were added to another cells before their exposure [26]. Although Belyaev and Scheglov *et al* did not totally exclude the chemical mechanism of the effects observed, they consider the electromagnetic hypothesis more suitable for explanation of the type of cellular communication [25,26,28].

Quite recently, similar effects of intercellular communication were observed in response to ionizing radiation [29]. *E. coli* cells were treated with ionizing radiation in the range of 0.1 cGy-1Gy, and cellular lysates were assayed for GCS with the use of AVTD method. Alipov *et al* found that the values of relative viscosity were greater at the greater cell density (4×10^{-8} cell/mL vs 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-rays exposure [29].

It should also be noted that the analogous cooperative cellular response to ionizing radiation was found in mammalian cells, and it was regarded as the "bystander effect" [30-36]. Although little is known about the precise mechanisms of the bystander effects, it is reasonable to propose that the phenomenon is rather universal and its mechanisms are similar to those for bacteria.

This review has tried to summarize the current knowledge on this controversial topic. Unfortunately, the experiments considered above do not allow formulation of any specific theory based on rigorously proven facts. Although some authors claimed that light [6,8,10,12,13] or sound [9,14,15] were responsible for the effects of remote bacterial communication, the researchers did not seriously consider whether there might be alternative explanations for the findings reported. Moreover, none of the authors has taken into account the electromagnetic properties of glass devices used as it was made, for example, in Albrecht-Buehler's experiments on distant communication of BHK cells [37]. Also, there were no stringent controls for diffusion of signaling volatiles in experiments on distant bacterial communication. In this connection, the use of radioactive substances (eg, ³H-leucine or some others) would be helpful for elimination of chemical's transfer possibility during the experiments. Finally, it must be said that none of the research on bacterial communication via light or sound except for early Matsushashi's experiments [9] have been repeated outside of the original investigators' laboratories.

However, it is possible that such attempts were made but the researchers did not want to inform about their vain endeavors.

Concluding Remarks

the phenomenon of biocommunication via electromagnetic fields was shown also in experiments with onion roots [4], seedlings of garden radish and barley [38], bean seedlings [15], pollen of cherry and plum [39], rat tumor cells [40], amniotic and nephritic human cultures [41], BHK cells [37], fish eggs, embryos and larvae [42], beetles and daphnia [43]. Obviously, the mode of biocommunication is rather a universal phenomenon. Most likely, living organisms evolved complementary types of electromagnetic and chemical communication. Some organisms use light as signals, while others use sound. It is my deep conviction that biocommunication phenomena have a common explanatory basis, and therefore a more sustained effort should be made for designing studies, which incorporate empirical knowledge and technology of both biology and physics.

Turning back to bacteria, a few notes about how the different electromagnetic stimuli (light and sound waves) have an effect should be made. The ability of different microorganisms to produce UV light is a well-known fact [44-46]. At the same time, there is a lot of evidence for UV-associated mutations [47]. In this connection, the mechanisms of growth-stimulating effects of UV light, which has been postulated by some authors [6,8] remains an enigma. One possibility for it is the use of UV-A light for intercellular signaling, which mutational ability is, probably, less than those for UV-C and UV-B.

Concerning the visible light, it is becoming apparent that bacteria may use different photoacceptor molecules for light absorption. For *E. coli* cells, for example, it was shown that cytochrome *bd* and *bo* complexes might be the main photoacceptors, and the primary and secondary mechanisms of light stimulation are also discussed [48]. However, in the above experiments on distant bacterial communication via light [10,12,13], the exact link between the some wavelength and biological response is absent. Therefore, the use of color filters would be helpful for specification of wavelength-dependent effects of visible light.

Still less is known about the possible role of infrared light, high frequency and low frequency electromagnetic fields to be signal carrier in microbial communication. For example, there is plenty of evidence to indicate that there are mechanisms for causing DNA covalent bond breakage [49,50].

Moreover, it was reported about the bactericidal effects of millimeter waves, which were connected with alteration in membrane permeability (F_0F_1 membrane bound protein complex is believed to play an important role in the process) and activation of lysogenic genes in bacterial genome [51]. However, how these electromagnetic fields of the aforementioned frequencies may take part in bacterial communication remains to be investigated.

Finally, the area of microbial communication via electromagnetic fields is totally undeveloped. Nevertheless, future research in the area will, undoubtedly, provide additional information for the understanding of different aspects of bacterial life, including bacterial pathogenicity and resistance to antimicrobials.

References

1. Kaprelyants AS Kell DK. Do bacteria need to communicate each other for growth? Trends Microbiol 1996;4:237-42.
2. O'Toole G, Kaplan HB, Kolter R. Biofilm formation as microbial development. Annu Rev Microbiol 2000;54:49-79.
3. Miller, MB, Bassler BL. Quorum sensing in bacteria. Annu Rev Microbiol 2001;55:165-99.
4. Gurvitch AG. Das Problem der Zellteilung Physiologisch Betrachtet: Berlin; Springer-Verlag; 1926.
5. Rahn O. Invisible Radiations of Organisms: Berlin; Borntraeger; 1936.
6. Nikolaev YuA. Distant interaction between bacterial cells. Microbiologiya [Russian] 1992;61:1065-71.
7. Flavier AB, Ganova-Raeva LM, Schell MA, Denny TP. Hierarchical autoinduction in *Ralstonia solanacearum*: control of acyl-homoserine lactone production by a novel autoregulatory system responsive to 3-hydroxypalmitic acid methyl ester. J Bacteriol 1997;179:7089-97.
8. Wainwright M, Kilham K, Russel C, Gravstone J. Partial evidence for the existence of mitogenetic radiation. Microbiology 1997;143:1-3.
9. Matsushashi M, Pankrushina AN, Endoh K, Watanabe H, Mano Y, Hyodo M, Fujita T, Kunugita K, Kaneko T, Otani S. Studies on carbon material requirements for bacterial proliferation and spore germination under stress conditions: a new mechanism involving transmission of electromagnetic signal. J Bacteriol 1995;177:688-93.
10. Nikolaev YuA. Distant interaction in the bacterium *Pseudomonas fluorescens* as a factor of adhesion regulation. Microbiologiya [Russian] 2000;69:356-61.
11. Nikolaev YuA. Regulation of the adhesion of *Pseudomonas fluorescens* cells to glass by extracellular volatile compounds. Microbiologiya [Russian] 2000;69:352-5.
12. Trushin MV Studies on distant regulation of bacterial growth and light emission. Microbiology 2003;149:363-8.
13. Trushin MV Culture-to-culture electromagnetic interactions causes the alteration in red and infrared light stimulation of *Escherichia coli* growth rate. J Microbiol Immunol Infect 2003; 37:149-52.
14. Matsushashi M, Shindo A, Ohshima H, Tobi M, Endo S, Watanabe H, Watanabe H, Pankrushina AN. Cellular signals

AQ1 = "photoreceptor"?

- regulating antibiotic sensitivities of bacteria. *Microb Drug Resist* 1996;2:91-3.
15. Matsuhashi M, Pankrushina AN, Takeuchi S, Ohshima H, Miyoi H, Endoh K, Murayama K, Watanabe H, Endo S, Tobi M, Mano Y, Hyodo M, Kobayashi H, Kaneko T, Otani S, Yoshimira S, Harata A, Sawada T. Production of sound waves by bacterial cells and the response of bacterial cells to sound. *J Gen Appl Microbiol* 1998;44:49-55.
 16. Kandel ER, Schwartz JH, Jessel TM. Principles of neural science 3rd edition: Norwalk, Connecticut: Appleton and Lange; 1991.
 17. Norris V, Hyland GJ. Do bacteria sing? Sonic inter-cellular communication between bacteria may reflect electromagnetic intra-cellular communication involving coherent collective vibrational modes that could integrate enzyme activities and gene expression. *Mol Microbiol* 1997;24:879-80.
 18. Fröhlich H. Long-range coherence and energy storage in biological systems. *Int J Quantum Chem* 1968;2:641-52.
 19. Volkov SN, Kosevich AM. Theory of low-frequency vibrations in DNA macromolecules. *J Biomol Struct Dyn* 1991;8:1069-83.
 20. Lisy V, Miskovsky P, Schreiber P. On a simple model of low-frequency vibrations in DNA macromolecules. *J Biomol Struct Dyn* 1996;13:707-16.
 21. Hengge-Aronis R. Back to log phase: sigma S as a global regulator in the osmotic control of gene expression in *Escherichia coli*. *Mol Microbiol* 1996;21:887-93.
 22. Hengge-Aronis R. Stationary phase gene regulation: what makes an *Escherichia coli* promoter sigmaS-selective? *Curr Opin Microbiol* 2002;5:591-5.
 23. Heal RD, Parsons AT. Novel intercellular communication system in *Escherichia coli* that confers antibiotic resistance between electromagnetically separated populations. *J Appl Microbiol* 2002;92:1116-22.
 24. Belyaev IYa, Alipov YeD, Scheglov VS, Polunin VA, Aizenberg OA. Cooperative response of *Escherichia coli* cells to the resonance effect of millimeter waves at super low-intensity. *Electro-Magnetobiol* 1994;13:53-66.
 25. Belyaev IYa, Alipov YeD, Matronchik AYU, Radko SP. Cooperativity in *E. coli* cell response to resonance effect of weak extremely low frequency electromagnetic field. *Bioelectrochem Bioenerg* 1995;37:85-90.
 26. Belyaev IYa, Alipov YeD, Matronchik Ayu. Cell density dependent response of *E. coli* cells to weak ELF magnetic fields. *Bioelectromagnetics* 1998;19:300-9.
 27. Belyaev IYa, Scheglov VS, Alipov YeD, Ushakov VD. Nonthermal effects of extremely high-frequency microwaves on chromatin conformation in cells *in vitro* – dependence on electromagnetic, physiological, and genetic factors. *IEEE T Microw Theory* 2000;48:2172-9.
 28. Shcheglov, VS, Alipov YeD, Belyaev Iya. Cell-to-cell communication in response of *E. coli* cells at different phases of growth to low-intensity microwaves. *Biochim Biophys Acta* 2002;1572:101-6.
 29. Alipov YeD, Shcheglov VS, Sarimov RM, Belyaev IYa. Cell-density dependent effects of low-dose ionizing radiation on *E. coli* cells. *Radiacionnaya biologiya. Radioekologiya [Russian]* 2003;43:1-6.
 30. Azzam EI, de Toledo SM, Goodning T, Little JB. Intercellular communication is involved in the bystander regulation of gene expression in human cells exposed to very low fluences of alpha particles. *Radiat Res* 1998;150:497-4.
 31. Zhou H, Randers-Pehrson G, Waldren CA, Vannais D, Hall EJ, Hei TK. Induction of a bystander mutagenic effect of alpha particles in mammalian cells. *Proc Natl Acad Sci USA* 2000; 97:2099-104.
 32. Mothersill C, Seymour C. Radiation-induced bystander effect: past history and future directions. *Radiat Res* 2000;155:759-67.
 33. Sawant SG, Randers-Pehrson G, Geard CR, Brenner DJ, Hall EJ. The bystander effect in radiation oncogenesis I. Transformation in C3H 10T1/2 cells *in vitro* can be initiated in the unirradiated neighbours of irradiated cells. *Radiat Res* 2001; 155:397-1.
 34. Belyakov OV, Malcolmson AM, Folkard M, Prise KM, Michael BD. Direct evidence for bystander effect of ionizing radiation in primary human fibroblasts. *Br J Cancer* 2001;84:674-9.
 35. Ward JF. The radiation-induced lesions which trigger the bystander effect. *Mutat Res* 2002;499:151-4.
 36. Sterreicher J, Prise KM, Michael BD, Vogt J, Butz T, Tanner J.M. Radiation-induced bystander effects. *Strahlenther Onkol* 2003;179:69-77.
 37. Albrecht-Buehler G. Rudimentary form of cellular "vision". *Proc Natl Acad Sci USA* 1992;89:8288-92.
 38. Kuzin AM. Rol prirodnogo radioaktivnogo fona i vtorichnogo biogennogo izlucheniya v yavlenii zhizni [The role of natural background radiation and secondary biogenic radiation in life]: Moscow: Nauka; 2002.
 39. Budagovskii AV, Turovtseva NM, Budagovskii IA. Coherent electromagnetic fields and remote cell interaction. *Biofizika [Russian]* 2001;46:860-6.
 40. Kirkin AF. Non-chemical (distant) interactions among cells in a culture. *Bifizika [Russian]* 1981;26:839-43.
 41. Kaznacheev VP, Mikhailova LP. Sverkhslabye izlucheniya v mezhkletochnykh vzaimodeistviyakh [Ultra-weak radiation in intercellular communication]: Novosibirsk: Nauka; 1981.
 42. Belousov LV, Burlakov AB, Luchinskaya NN. Statistical and frequency-amplitude characteristics of ultraweak emissions of the loach eggs and embryos under the normal conditions and during their optic interactions. I. Characteristics of ultraweak emission in normal development and the optic role of egg envelopes. *Ontogenez [Russian]* 2002;33:313-21.
 43. Chang JJ, Popp FA, Yu WD. Biocommunication and bioluminescence of *Lampyridae*. In: *Biophotonics Non-equilibrium and coherent systems in biology, biophysics and biotechnology*. Moscow: Bioinform Services Co.; 1995:267-280.
 44. Quickenden TI, Tilbury RN. Growth dependent luminescence from cultures of normal and respiratory deficient *Saccharomyces cerevisiae*. *Photochem Photobiol* 1983;37:337-44.
 45. Quickenden TI, Tilbury RN. An attempt to stimulate mitosis in *Saccharomyces cerevisiae* with the ultraviolet luminescence from exponential phase cultures of this yeast. *Radiation Res* 1985;102:254-63.
 46. Tilbury RN, Quickenden TI. Spectral and time dependence studies of the ultraweak bioluminescence emitted by the bacterium *E. coli*. *Photochem Photobiol* 1988;47:145-50.
 47. Davies, RJH. Ultraviolet radiation damage in DNA. *Biochem Soc Trans* 1995;23:407-18.
 48. Karu TI. Primary and secondary mechanisms of action of visible-to-near IR radiation on cells. *J Photochem Photobiol: B Biol* 1999;49:1-17.
 49. Kakita Y, Kashige N, Murata K, Kuroiwa A, Funatsu M, Watanabe K. Inactivation of *Lactobacillus bacteriophage* PL-1

- by microwave irradiation. *Microbiol Immunol* 1995;39:571-6.
50. Kakita Y, Funatso M, Miake F, Watanabe K. Effects of microwave irradiation on bacteria attached to the hospital white coats. *International J Occup Med Environ Health* 1999;12:123-6.
51. Trchunian A, Ogandzhanian E, Sarkisian E, Gonian S, Ogenesian A, Ogenesian S. Membranotropic effects of electromagnetic radiation of extremely high frequency on *Escherichia coli* cells. *Biofizika* [Russian] 2001;46:69-76.



LIPPINCOTT
WILLIAMS & WILKINS

Journal of Microbiology, Immunology and Infection
Volume 36 no. 3

Reprint Order Form

I would like to order reprints for my articles:

Title _____

Copies & Charges (please ✓ the appropriate box):

No. of pages	50 cps	100 cps	150 cps	200 cps	250 cps
1-8	<input type="checkbox"/> USD240	<input type="checkbox"/> USD280	<input type="checkbox"/> USD317	<input type="checkbox"/> USD351	<input type="checkbox"/> USD382
9-16	<input type="checkbox"/> USD352	<input type="checkbox"/> USD406	<input type="checkbox"/> USD457	<input type="checkbox"/> USD505	<input type="checkbox"/> USD550
17-24	<input type="checkbox"/> USD484	<input type="checkbox"/> USD550	<input type="checkbox"/> USD614	<input type="checkbox"/> USD676	<input type="checkbox"/> USD736

No. of pages	300 cps	350 cps	400 cps	450 cps	500 cps
1-8	<input type="checkbox"/> USD412	<input type="checkbox"/> USD441	<input type="checkbox"/> USD469	<input type="checkbox"/> USD496	<input type="checkbox"/> USD519
9-16	<input type="checkbox"/> USD593	<input type="checkbox"/> USD634	<input type="checkbox"/> USD673	<input type="checkbox"/> USD710	<input type="checkbox"/> USD745
17-24	<input type="checkbox"/> USD792	<input type="checkbox"/> USD845	<input type="checkbox"/> USD895	<input type="checkbox"/> USD942	<input type="checkbox"/> USD987

* Additional charge of USD70 per page for color illustrations

Please deliver to:

Name: _____
 Address : _____

 Tel: _____ Fax: _____
 E-mail: _____

Payment Options:

I enclose payment _____
 (If pay by cheque, please make payable to **Lippincott Williams & Wilkins Asia Ltd.**)

Please charge my credit card:

* American Express / Visa / Mastercard / OTB card *delete as applicable

Card No.: _____

Signature _____ Expiry date _____

Please return this form by fax and make your payment before **July 30, 2003** to:
Lippincott Williams & Wilkins Asia Ltd.



Lippincott Williams & Wilkins Asia Limited
 Suite 907-910, Wharf T & T Centre, Harbour City, 7 Canton Road, Tsim Sha Tsui, Kowloon, Hong Kong
 Tel: (852) 2610 2339 • Fax: (852) 2421 1123 • E-mail: weserve@lww.com.hk • LWW.com