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EXPERIMENTAL ARTICLES

Effect of Mechanical Vibrations on the *lon* Mutant of *Escherichia coli* K-12

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Abstract—It was found that, depending on their frequency, mechanical vibrations (MVs) can either stimulate (4 Hz) or inhibit (50 Hz) the growth and the division of the *lon* mutant of *Escherichia coli* K-12. Similar effects were observed when the MV-treated nutrient medium was inoculated with untreated mutant cells. MVs enhanced the motility of mutant cells and the fragmentation of filament cells always present in the populations of *lon* mutants.

Key words: mechanical vibrations, *Escherichia coli*, *lon* mutant, cell division, motility.

There is vast literature devoted to the effect of physical and chemical factors on the activity of various organisms, whereas information on the effect of mechanical vibrations (MVs) of different frequencies on living organisms is limited.

Earlier, it was shown that physiological saline solution exposed to 4-Hz vibrations diminishes the inhibitory action of acetylcholine on the heart contraction amplitude in the snail *Helix pomatia* [1]. MVs were also found to induce frequency-dependent changes in the electrical conductivity of water, which were most pronounced when the water was exposed to 4-Hz MVs [2]. It was suggested that MVs affect living organisms indirectly by changing the physical and chemical properties of water.

The study of the action of MVs on the activity of microorganisms is of particular interest, since microorganisms are very sensitive to various physical and chemical factors. In particular, the *lon* mutant of *Escherichia coli* K-12 was found to be highly sensitive to ionizing radiation, UV light, and the inhibitors of DNA synthesis. These factors inhibit cell division at the stage of formation of the intracellular wall (septum), so the affected cells grow as long filaments incapable of forming macrocolonies on agar media [3, 4]. On the other hand, other physical and chemical factors are able to recover the normal division ability of the *lon* mutants [3–6].

We present here the results of investigation into the effect of MVs on the activity of the *lon* mutants of *E. coli* K-12.

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MATERIALS AND METHODS

Bacterial strains. *Escherichia coli* strains K-12 and CA 154 (*thi*, *lacZ*) were obtained from the Collection of Microbial Cultures at the Institute of Genetics and Selection of Industrial Microorganisms (GOSNIIGENETIKA, Moscow); the slime-producing strain MUC 154-2 (*thi*, *lacZ*, *lon*) was derived from strain CA 154 in our laboratory. To prevent the unwanted effect of slime on the rheological properties of the media, the non-slime-producing mutant NM 154-9 (*thi*, *lacZ*, *lon*, *muc*) was derived from strain MUC 154-2 using the virulent phage M59 specific to *lon* mutants [7].

Nutrient media used in this study were as follows: nutrient broth enriched (NBE); nutrient broth enriched standard 1 (NBE 1), purchased from Serva; bactopeptone broth (1% Difco peptone); and nutrient agar enriched (NAE), representing NBE with 1.5% Difco agar.

To study the mechanism through which MVs affect bacterial cells, we performed two sets of experiments. In the first set of experiments, 0.5 ml of the exponentially growing culture (3×10^{-8} cells/ml) was added to 3.5 ml of NBE and the suspension was exposed to MVs of different frequencies (4, 10, and 50 Hz) at an intensity of 90 dB for 30 min. Unexposed culture served as the control.

In the second set of experiments, 0.5 ml of the same culture was added to 3.5 ml of NBE, which was pretreated with MVs, and the suspension was incubated at 37°C. In these experiments, the culture mixed with untreated medium served as the control.

In both sets of experiments, the concentration of cells in suspensions was evaluated by plating them on

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complete agar medium at certain time intervals. The home-built device [1, 2], whose block diagram is given in Fig. 1, generated vibrations with frequencies from 3 to 2500 Hz and intensities up to 90 dB and transmitted them to test tubes *1* (10 × 50 mm) filled with the medium to be vibrated and safely fixed to radiator *3*. Vibrator *4*, which was activated by a G3-118 generator of sinusoidal signals (*7*) manufactured in the Russian Federation, generated vibrations in a vertical plane at a specified frequency and intensity. The vibrations were transmitted to the medium inside the test tubes with an insignificant power loss. Power amplifier *6* served to match the high-resistance output of generator *7* with the low-resistance input of vibrator *4*. Vibrational frequency was controlled by a Ch3-47A frequency meter (*8*) manufactured in the Russian Federation. The intensity of vibrations was controlled by special unit *5*, which received signals from probe *2* situated on the radiator *3* of vibrator *4*. This control system allowed the intensity of vibrations to be maintained at a specified level at all the frequencies used, including the resonance frequency (somewhat higher than 200 Hz), as well as to avoid the unwanted effects of mechanical and hydrodynamic resonances. The nonlinearity of vibrations at an intensity of 90 dB did not exceed 2%.

To avoid the aeration of samples during vibrational treatment, the tubes were 95% full of the medium and were tightly sealed.

The morphology of cells and their responses to MVs of different frequencies were examined in a Binocular phase-contrast microscope (Poland) at a magnification of 1400×. The motility of cells was measured using two communicating cuvettes (10 × 10 × 40 mm), which are commonly employed for measuring optical densities with an SF-46 spectrophotometer (Fig. 2). One of the cuvettes contained nutrient medium with the MV-pretreated cells (in the control experiment, cells were untreated). The other cuvette contained the same nutrient medium but without cells; changes in the optical density of this medium were recorded at 540 nm for 60 min. The media in the cuvettes were linked through a polyethylene tubing 2 mm in diameter filled with the nutrient medium. Cells from the first cuvette could pass to the nutrient medium of the second cuvette, thereby increasing its optical density. The small sizes of the tubing and cuvettes served to minimize hydrodynamic flows in them. The reproduction of the cells that arrived to the second cuvette could not significantly influence the result of the experiment, since the conditions of cell reproduction in the both cuvettes were the same. Experiments were performed at 23–25°C at an atmospheric pressure of 87.65–87.95 kPa.

The results are presented as the means of 5 replicated experiments. Significance levels, assessed in terms of Student's *t*-test, arithmetic means, and standard deviations were calculated using the Sigma Plot 1.02 software program (Jandel Corporation).

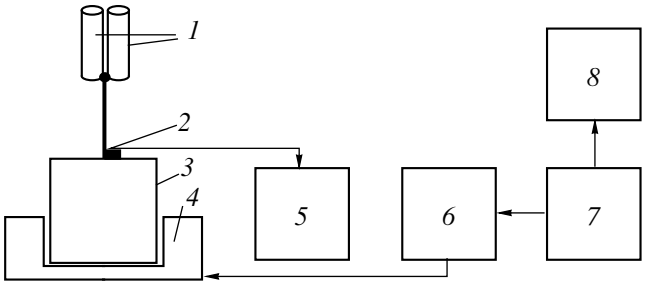

Fig. 1. Block diagram of the laboratory device for the generation and transmission of vibrations to the medium to be treated: (*1*) glass tubes with the medium; (*2*) probe; (*3*) radiator; (*4*) vibrator; (*5*) unit for measuring and controlling the intensity of vibrations; (*6*) power amplifier; (*7*) driving generator of sinusoidal vibrations; and (*8*) frequency meter.

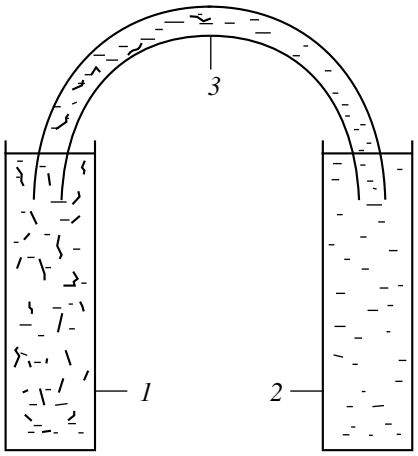

Fig. 2. Communicating cuvettes for the estimation of cell motility: (*1*) cuvette with the culture tested for cell motility; (*2*) cuvette with the nutrient medium whose optical density, measured at 540 nm, increased as motile cells passed from cuvette *1*; (*3*) tubing with the nutrient medium linking the cuvettes.

RESULTS AND DISCUSSION

Experiments showed that the exponential-phase cells of the *lon* mutant *E. coli* NM 154-9 responded differently to 30-min treatments with different vibrational frequencies. Namely, 4-Hz vibrations induced a threefold increase in the number of cells as compared to the control ($P < 0.01$); 10-Hz vibrations did not induce any significant change in the number of cells; and 50-Hz vibrations led to a 1.4-fold decrease ($P < 0.05$) in the number of cells capable of forming colonies on the test agar medium (Fig. 3a).

After 3 h of incubation under stationary conditions (Fig. 3b), 4 Hz-exposed culture showed better growth than the control, 10-, and 50 Hz-exposed cultures. During such incubation, the concentration of cells exposed to 50-Hz vibrations decreased further by more than two times ($P < 0.01$). Bearing in mind that MVs can affect

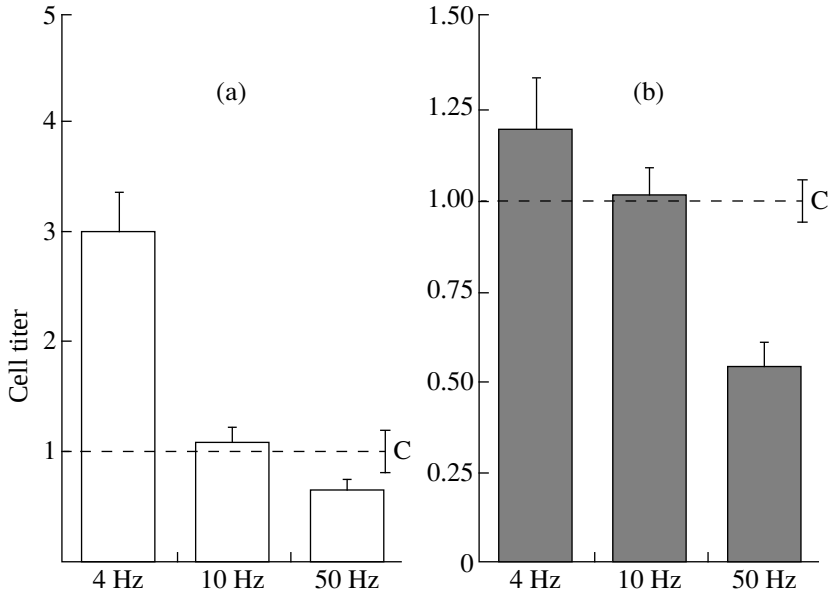

Fig. 3. Effect of the 0.5-h exposure of cells to different vibrations on their reproduction: (a) the cell concentration was estimated immediately after the ending of the MV treatment and (b) 3 h afterwards. The number of cells in the control culture (C) was arbitrarily taken to be unity.

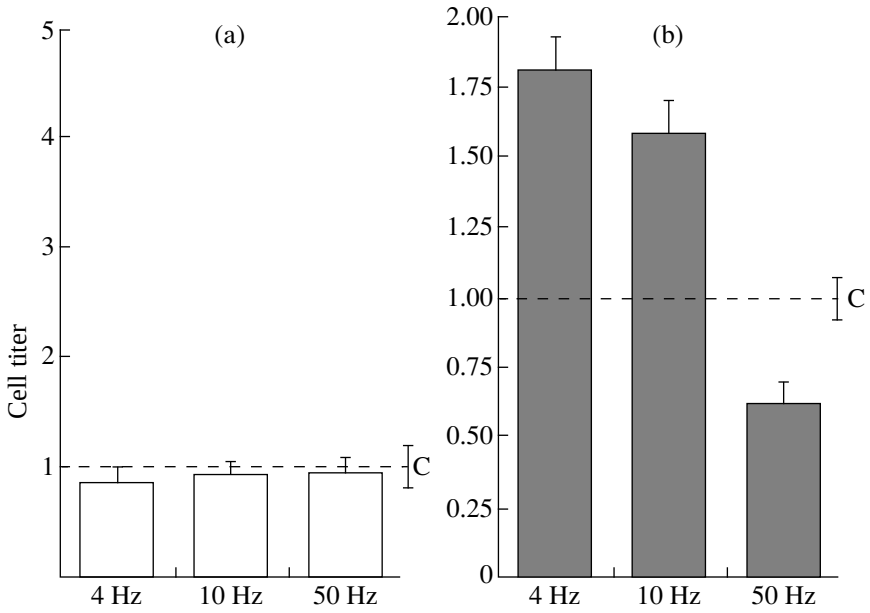

Fig. 4. Effect of the MV-pretreated nutrient medium on the reproduction of bacterial cells: the concentration of cells was estimated (a) 30 min and (b) 3 h after they were placed in the MV-treated medium. The number of cells in the control culture (C) was taken to be unity.

cells not only directly, but also indirectly (through changes in the medium), we attempted to evaluate the medium-mediated effect of MVs on the cells. For this, the NBE pretreated with MVs was inoculated with intact cells and incubated at 37°C (Fig. 4). After a 30-min incubation, the concentration of cells did not significantly change (Fig. 4a). However, after 3 h of incubation (Fig. 4b), the NBE pretreated with 4- and 10-Hz vibrations provided for, respectively, 1.75- and 1.5-fold better growth than the control medium ($P < 0.05$ for the experiments with both vibrational frequencies). At the same time, the NBE pretreated with 50-Hz vibrations inhibited the culture growth about twofold as compared to the control culture ($P < 0.05$).

The microscopic observations of the cells exposed to MVs either directly or indirectly (through the treat-

ment of the culture medium) showed a drastic increase in their motility. The 0.5-h treatment of cultures with 4- and 10-Hz vibrations stimulated the fragmentation of the cell filaments spontaneously formed in the *lon* mutant population.

Changes in the motility of cells exposed to MVs were also studied in the device described above by measuring the optical densities of cell suspensions in the cuvettes (Fig. 5). The measurements showed that the MV-treated cells more rapidly migrated from cuvette *1* to cuvette *2* than did the control cells. The migration rate of cells was particularly high 60 min after their exposure to 10-Hz vibrations ($P < 0.01$).

As follows from the experimental data presented, MVs substantially influence the growth rate and the division of bacterial cells through both the direct and indirect (medium-mediated) action on the cells. In both cases, MVs either stimulate (4- and 10-Hz vibrations) or inhibit (50-Hz vibrations) the growth of *E. coli* cells. When the cells were exposed to 4-Hz vibrations for 30 min, the culture grew three times as well as the control culture. Hence, the culture treated with 4-Hz vibrations for 30 min at 37°C produced 2–3 new cell generations, while the untreated culture produced no more than one new cell generation over the same time period.

It is known that cell reproduction is limited by the rate of DNA replication, since the rate of the cell wall synthesis during the segregation of daughter cells is almost constant [8]. Therefore, enhanced cell reproduction may be due to an increase in the number of replicative folks (RFs) and the transfer of replicating DNA to daughter cells. In our studies, the rapid growth of cells exposed to 4-Hz vibrations was probably associated with the accelerated cell division due to an increased number of RFs per chromosome.

The two times poorer growth of the culture treated with 50-Hz vibrations for 30 min in comparison with the control culture was most likely due to the slow division of cells but not to their death. The twofold difference in the densities of the MV-treated and control cultures did not increase in the next 3 h of their incubation, indicating that the effect of MVs on cells does not develop.

Of interest is the fact that the MV-treated nutrient medium can also influence the culture growth. The effect of the MV-treated medium is not immediate and develops during the growth and division of inoculated cells. High concentrations of cells in the medium (such as 10^7 cells/ml) can diminish the effect of MVs on the culture. This explains why the effect of the MV-treated growth medium with inoculated cells was not so pronounced after 3 h of incubation.

It should be noted that filament cells, which are 50 to 100 times as long as normal cells and comprise 1–2% of the population of *lon* mutants, can also contribute to the increase in the culture density in response to exposure to MVs. The division of filament cells exposed to 4-Hz vibrations was found to increase the culture density by 1.5–2.0 times.

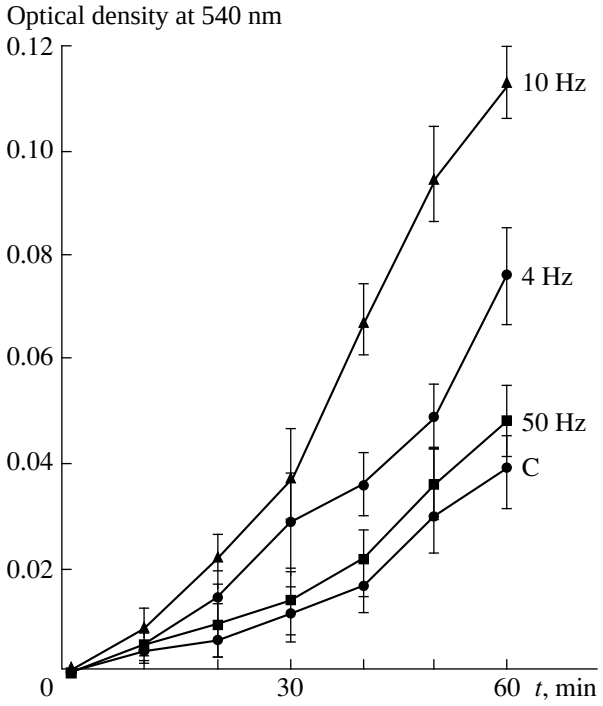

Fig. 5. Changes in the optical density of medium in cuvette *2* caused by the passage of motile cells from cuvette *1* with the MV-treated or control (C) culture.

In the *lon* mutants, cell division is inhibited by the SulA protein of the SOS regulon, whose synthesis is induced in response to UV irradiation and the action of radiomimetics [9]. This protein prevents the formation of the FTsZ ring, a precursor of the cell septum [10]. The suppressed cell division is recovered as soon as the Lon proteinase cleaves the SulA protein [8]. The *lon* gene is a component of the HTP regulon, which is induced in response to high temperatures [9]. It can be suggested that MVs stimulate the residual Lon proteinase present in the *lon* mutants [11], which cleaves SulA and thus contributes to the recovery of filament division. However, the possibility cannot be excluded that MVs break the FtsZ–SulA bonds and thus induce the synthesis of the cell septum. This can also explain the weaker effect of 4-Hz vibrations when their action on cells is mediated by the medium (Fig. 4b).

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