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The synergistic effect of ultrasound and γ-radiation on *Lactobacillus casei* bacteria in the presence of a theraphthal sonosensitizer

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S1. Experimental details

1. Bacterial culture

For the experiments, lyophilized cultures of bacteria *Lactobacillus casei*, "AiBi" series Lb 3.02 were used, which were thawed before the experiment and left in warm (37 °C) skim milk for 1 hour to activate the culture.

2. Theraphthal

Sodium salt of cobalt 4,5-octacarboxyphthalocyanine (GNC NIOPIK, Russian Federation, RF Patent 2106146, 1998; registration number P T001057/01-2002).

3. Cultivation of bacteria

For the cultivation of *Lactobacillus casei* bacteria, skimmed milk or whey (skimmed milk) was used. Bacteria were cultured on a gel medium. The medium was prepared immediately before the experiments. To do this, 15% agarose by weight was dissolved in skim milk when heated, sterilized, poured into sterile plastic Petri dishes and cooled to room temperature. The prepared medium in dishes was stored in the refrigerator. Cultivation of bacteria was carried out at a temperature of 37 °C [S1].

For the experiments, a suspension of bacterial cells was prepared in tap water sterilized by boiling at the required dilution. The concentration of cells in the suspension for experiments was 2300-4000 cells/mL.

The effectiveness of the effect was assessed by the ratio of the number of colony-forming units (CFU) in the control suspension and in the experiments. To do this, 1 mL of the control suspension was seeded onto the gel medium and after 24 hours the number of CFU was calculated as the number of colonies formed on the medium. Screening and counting of CFUs in experiments were carried out similarly. Cultivation of bacteria was carried out in an air thermostat at a temperature of 37 °C. The result of bacterial growth was assessed after 24 hours. All experiments were carried out in three series. Mean values and standard deviations were calculated. The final result was evaluated in the control/experiment ratio.

4. Exposure to ionizing radiation

γ-Irradiation was carried out with the set of γ-emitting ^{137}Cs sources located uniformly around the sample. Resulting dose rate was 2.3 Gy/min. Samples 10-30 mL in volume were irradiated at room temperature in glass vials.

5. Ultrasonic exposure

Ultrasonication was carried out in a thermostated (24 °C) cell through ultrasound conductive medium, with intensity of 2 W/cm^2, frequency of 0.88 MHz. The volume of the sonicated medium was 10 mL.

6. Combined effects of ultrasound and ionizing radiation

The combined effects of γ-radiation and ultrasound on objects were carried out, alternating in different orders, under standard conditions, ultrasound and γ-irradiation, with a fixed time separating both procedures.

7. Scanning electron microscopy

Cell suspensions were applied to track nuclear filters and dried in air. Fragments of filters with cells were mounted on a stage. After this, the surface of the samples was coated with gold (layer thickness 20 nm) in an IB-3 ion coating unit (EIKO). The material was examined in a Cam Scan scanning electron microscope (Hitachi) at an accelerating voltage of 15 kV and a working magnification from 1000 to 10000.

8. Assessment of the toxicity of theraphthal to bacteria

The introduction of theraphthal at concentrations of 10^{-4} and 10^{-5} M into a cell suspension and the determination of CFU units when screening out an aliquot of the suspension did not affect the number of CFU bacteria on the gel medium. The toxicity of theraphthal to bacterial cultures was also assessed. The bacteria were incubated in a liquid medium (salt medium M9 with glucose) with the addition of theraphthal at a concentration of 10^{-3} M for 5 days. Twice a day, after 8 and 16 hours, the incubation solution was replaced in order to prevent the influence of an increase in the density of bacterial cells on the rate of their growth and division, as well as changes in the quality of the medium due to metabolites released by the cells. As a criterion for the state of the culture, we used the specific growth rate, which was calculated using the formula $r=dN/(Ndt)$ and the average specific growth rate per day was calculated. The change in specific growth rate was insignificant, indicating the absence of a toxic effect of theraphthal on bacterial cells.

References

S1 *Praktikum po mikrobiologii (Workshop on Microbiology)*, ed. A. I. Netrusov, Academia, Moscow, 2005 (in Russian).