CONFERENCE MATERIALS

The International Scientific and Practical Conference "MODERN ASPECTS OF MICROBIOLOGY, VIROLOGY, AND BIOTECHNOLOGY IN WARTIME AND POST-WAR PERIOD"

November 15-16, 2023 Kyiv, Ukraine

> D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine

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EXTRA AND INTERCELLULAR SYNTHESIS OF SELENIUM NANOPARTICLES USING PROBIOTIC BACTERIA

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Introduction. One major health problem worldwide is Selenium deficiency [1]. Microorganisms biosynthesize selenium nanoparticles (Nano-Se) during the transformation of selenite and selenate, but the metabolic pathways by which phylogenetically related *Lactobacillus* and *Bacillus* species bio-convert selenite remain unclear.

However, *Bacillus subtilis* and *Lactobacillus* strains are probiotic microorganisms, which are considered to be selenium-rich microorganisms, that are capable of transforming sodium selenite to elemental selenium.

The goal of this study was to conduct a screening among probiotic strains of *Lactobacillus* and *Bacillus* species capable to reduce sodium selenite with the formation of biogenic Nano-Se for using as dietary supplements.

Method. Probiotic cultures *L. casei IMB B-7280*, *L. plantarum IMB B-7679*, *B. subtilis* IMB B-7392, *B. subtilis* B-7393, *B. clausii* were grown in Erlenmeyer's flasks of 750 mL with shaking at 240 rpm for 48 hours at a temperature of 30 °C and pH 6.5 on a nutrient medium enriched with Na₂SeO₃. For the cultivation of *Lactobacillus* species and aerobic spore-forming bacteria, MRS and Nutrient Broth No.1 were used, respectively. A visual assessment of the color change of the nutrient culture medium was carried out under the conditions of its enrichment with 3 ppm Se in the composition of sodium selenite. Visualization of the formation of biogenic Nano-Se was performed by using transmission electron microscopy (TEM).

Results: An original method of using probiotic *Lactobacillus* species in the biosynthesis of nanoselenium (Nano-Se) is proposed. The ability of *L. casei* IMB B-7280 and *L. plantarum* IMB B-7679 cultures to grow on MRS nutrient medium in the presence of 3 ppm Se in the form of Na₂SeO₃ was shown.

Enrichment of the culture medium with sodium selenite under the conditions of culture growth revealed the ability of *Lactobacillus* species to reduce Se (IV) oxyanions and the formation of an orange color.

A change in the color of the medium under the influence of Na_2SeO_3 during the cultivation of *Lactobacillus* species was noted in the case of the middle of the exponential growth phase (8-10 h), and an extension of the lag phase (delayed reproduction) was observed.

Thus, in the presence of 3 ppm Se, the biomass concentration of *L. casei* IMV B-7280 was $(8.4 \pm 0.4) \times 10^7$ CFU/mL and in the absence of Na₂SeO₃ $(6.2 \pm 0.1) \times 10^8$ CFU/mL. While in the presence of 3 ppm Se, the biomass concentration of L. plantarum IMB B-7679 was $(2.3 \pm 0.4) \times 10^6$ and which was in the absence of Na₂SeO₃ $(4.5 \pm 0.2) \times 10^7$ CFU/mL. Both research strains showed the ability to reduce Se⁴⁺ in the internal compartments of the cell with the formation of biogenic Nano-Se of different sizes, with their subsequent release into the extracellular space of *Lactobacillus* species. *Lactobacillus*-derived Nano-Se were observed using Transmission Electron Microscopy (TEM). TEM analysis revealed electron-dense spheres 120–150 nm in size.

Among the microorganisms of the genus *Bacillus*, some strains of *B. subtilis* IMB including B-7392 and B-7393, have found an ability to transform sodium selenite, with the formation of biogenic Nano-Se.

The ability to reduce selenite into selenium nanoparticles was demonstrated in *B. clausii* for the first time [2].

But the formation of Nano-Se effects on the extension of the logarithmic phase of growth of crops and is accompanied by a decrease in the concentration of biomass aerobic spore-forming bacteria.

According to TEM data, the extracellular pathway of Nano-Se formation by cultures of *B. subtilis* IMB B-7392, B-7393, *B. clausii* was proven. It should be noted that the extracellular biosynthesis of Nano-Se is preferable to the intracellular synthesis of Nano-Se, as it occurs outside the bacterial cell and it is cheaper to obtain Nano-Se.

Nanostructures based on selenium nanoparticles and probiotics with proven safety can b targeted and effectively used to increase the productivity and viability of poultry [3]. Furthermore, Nano-Se synthesized by microorganisms are already used in various fields -]from biomedicine to the production of renewable energy [4].

Thus, Nano-Se of bacterial origin exhibit semiconductive and photoconductive properties and have unique optical and photoluminescence properties (5,6).

The green production of Nano-Se has developed to be an alternative to traditional physical and chemical methods of obtaining elemental selenium nanoparticles.

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