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BIONANOTECHNOLOGY OF SELENITE IONS RECOVERY INTO NANOSELENIUM BY PROBIOTIC STRAINS OF LACTOBACTERIA AND TOLERANCE OF LACTOBACTERIA TO SODIUM SELENITE

Green synthesis of nanoparticles (NPs) using living cells is a promising and new tool in bionanotechnology. Chemical and physical methods are used to synthesize NPs, but biological methods are preferred because of their environmentally friendly, clean, safe, cost-effective, simple, and efficient sources for high productivity and purity. ***Aim.*** *To investigate the processes of bioreduction of selenite ions into nanoselenium by probiotic strains of lactobacilli Lactobacillus plantarum IMV B-7679 and L. casei IMV B-7280.* ***Methods.*** *Cultivation of lactobacilli L. plantarum IMV B-7679 and L. casei IMV B-7280 was carried out in vials (500 cm^3) on a rotary shaker (220 rpm) at 30 °C for 2 days on the Man, Rogosa, and Sharpe (MRS) broth nutrient medium. Sodium selenite was additionally added to the environment in different concentrations from 1 to 30 ppm by Se. The number of viable bacterial cells in 1 mL of suspension was determined by the method of limiting dilutions in the case of sowing aliquots on a nutrient medium containing 0.2% agar-agar. Cultures of L. plantarum IMV B-7679 or L. casei IMV B-7280 were grown in the liquid MRS broth medium with low pH in the presence or absence of Na_2SeO_3. The concentration of sodium selenite ranged from 1 to 30 ppm by Se level. The number of microorganisms was determined by inoculation (0.1 mL of suspension) in dense media on cups with MRS agar medium, and the seeding dose was 10^7 cells/Petri dish. The tolerance of lactobacilli to the selenite ions was evaluated by the decrease in the number of CFU when sowing aliquots taken from culture samples grown in the presence or absence of selenite. The results of the experiments were presented in CFU and transferred to Log CFU/cm^3. The characteristics of Nano-Se were studied using transmission electron microscopy (TEM).* ***Results.*** *It was found that after 48 h incubation in*

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an MRS medium with the addition of sodium selenite from 1 to 30 ppm, the culture of L. plantarum IMV B-7679 was the most resistant. Thus, enrichment of the culture medium with 30 ppm of Se in the Na_2SeO_3 composition led to a decrease in the number of L. plantarum IMV B-7679 to 5.17 ± 0.09 Log CFU/cm^3 against 4.41 ± 0.11 Log CFU/cm^3 for L. casei IMV B-7280 in the control. The use of lower concentrations (1—3 ppm of Se in Na2SeO3) did not affect the change in morphology and cultural properties of L. plantarum IMV B-7679. The ability of L. casei IMV B-7280 and L. plantarum IMV B-7679 cultures to grow on MRSA nutrient medium in the presence of 3 ppm Se was shown. Higher tolerance to sodium selenite was found for L. plantarum IMV B-7679. Thus, increasing the concentration to 30 ppm of Se in the form of Na_2SeO_3 led to a decrease in the viability of only the culture of L. casei IMV B-7280. That is, the studied lactobacilli showed different ability to grow in the presence of selenite ions. The formation of round electron-dense granules sizing from 30 nm to 250 nm was observed using TEM. Both probiotic strains showed the ability to restore selenite ions with the accumulation of intracellular Nano-Se and the release of Nano-Se into the culture medium, which was accompanied by color shifts from yellowish to red-brown. The partial destruction of L. casei IMV B-7280 cells under the influence of oxyanions was revealed, which was accompanied by the release of culture-synthesized electron-dense Nano-Se particles. ***Conclusions.*** *The optimal conditions for the growth of L. plantarum IMV B-7679 and L. casei IMV B-7280 in the presence of Na_2SeO_3 were established, and it was proved that lactobacilli have different abilities to grow in the presence of selenite ions. The obtained data indicate that the investigated probiotic strains showed the ability to restore selenite ions along with the accumulation of intracellular Nano-Se and the release of Nano-Se into the culture medium.*

Keywords: *green synthesis, sodium selenite, selenium nanoparticles, probiotic strains, L. plantarum IMV B-7679, L. casei IMV B-7280, TEM.*

The statement of the problem and analysis of the latest research. Green synthesis of nanoparticles (NPs) using living cells is a promising and new tool in bionanotechnology. Chemical and physical methods are used to synthesize NPs, but biological methods are preferred owing to their environmentally friendly, clean, safe, cost-effective, simple, and efficient sources for high productivity and purity. Intra- or extracellular biosynthesis of NPs can be achieved by many biological entities, including bacteria, fungi, yeast, algae, actinomycetes, and plant extracts [1].

Selenium is an irreplaceable trace element that plays a vital role in the growth and development of living organisms. It is of great interest as a therapeutic factor without significant side effects at optimal doses. Selenium nanoparticles can be obtained by physical, biological, and chemical methods [2]. Biogenic green synthesis of nanoparticles shows an advantage compared to the other methods as it is environmentally friendly, quite reproducible, and easily available. Nanoparticles obtained by the method of green synthesis are able to be metabolized biologically [3, 4]. Some bacteria can reduce selenite/selenate to nanoselenium, so they are used as nanofactories that provide a new approach to the reduction of metalloid ions and the synthesis of materials with unique properties [1, 5].

So, nitrate-reducing bacteria *Bacillus ryziterrae* sp. were used for the intracellular reduction of selenite to selenium nanoparticles [6]. Recently, the synthesis of selenium nanoparticles by *Lactobacillus casei* ATCC 393 was investigated, and the formation of red selenium nanoparticles with a size of 50-80 nm, which accumulate in the bacterium intracellularly, was established [7].

As known, bacteria reduce SeO_3^{2-} to insoluble elemental selenium (Se^0) with the help of selenate reductases [8]. However, the ability to form biogenic nano-Se particles is a strain characteristic of the culture. Thus, lactobacilli have oxidoreductase systems for regulating the concentration of metals and metalloids. The penetration of metals into the bacterial cell occurs with the help of the membrane system of electron transport and reducing enzymes. Selenate can have two possible metabolic pathways: dissimilatory reduction of selenates accompanied by the formation of elemental selenium and assimilatory reduction of selenium accompanied by the formation of volatile selenium and/or organic sele-

nium compounds. The final products of dissimilatory reactions in various types of bacteria are red biogenic Nano-Se particles [9, 10].

The purpose of the study. The purpose of these experiments was to study the processes of bioreduction of selenite ions by probiotic strains of lactobacilli *L. plantarum* IMB B-7679 and *L. casei* IMV B-7280, which can be used for the green synthesis of selenium nanoparticles and their prospects for biotechnological application. The strains were obtained from the collection of microorganisms at the Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine.

Materials and methods. Cultivation of lactobacilli was carried out in vials (500 cm^3) on a rotary shaker (220 rpm) at 30 °C for 2 days on the nutrient medium Man, Rogosa, and Sharpe (MRS) broth (Condalab, Spain). Sodium selenite was added to the environment in different concentrations from 1 to 30 ppm by Se. The number of viable bacterial cells in 1 cm^3 of suspension was determined by the method of limiting dilutions in the case of sowing aliquots on a nutrient medium containing 0.2% agar-agar [11]. Cultures of *L. plantarum* IMV B-7679 or *L. casei* IMV B-7280 were grown in a liquid MRS broth medium with low pH in the presence or absence of Na_2SeO_3. The concentration of sodium selenite ranged from 1 to 30 ppm by Se level. The number of microorganisms was determined by inoculation of 0.1 cm^3 of suspension in dense media on Petri dishes with MRS agar, and the seeding dose was 10^7 cells/Petri dish. The tolerance of lactobacilli to the influence of selenite ions was determined by the decrease in the number of CFU when sowing aliquots taken from culture samples that grew in the presence of selenite or in its absence. The results of the experiments were presented in the CFU and transferred to the Log CFU/cm^3. The characteristics of Nano-Se were studied using transmission electron microscopy (TEM) with a JEM-1400 electron microscope (Japan).

Results. Under the influence of sodium selenite in the case of enrichment of the culture medium, a change in the color of the culture medium of lactobacilli was observed. Table 1 presents the results of the study of the influence of sodium selenite by the level of Se (ppm) in its composition on the growth of lactobacilli under cultivation conditions for 48 h at 30 °C.

It was found that after 48 h of incubation on the MRS medium with the addition of sodium selenite from 1 to 30 ppm, the culture of *L. plantarum* IMV B-7679 was the most resistant. Enrichment of the culture medium with 30 ppm of Se in Na_2SeO_3 led to a decrease in the number of *L. plantarum* IMV B-7679 — to 5.17 ± 0.09 Log CFU/cm^3 against 4.41 ± 0.11 Log CFU/cm^3 for *L. casei* IMV B-7280.

A change in the color of the culture medium of microorganisms growing in the presence of Na_2SeO_3 to different shades of red-brown is a characteristic sign of the formation of biogenic nanoselenium (Nano-Se) [12].

Determination of the impact of selenite ions on the growth of lactobacilli. To study the influence of selenium in an ionic form on the growth of *L. plantarum* IMV B-7679, the growth curve of this strain was built under normal condi-

Table 1. **The influence of sodium selenite by the level of Se (ppm) in its composition on the growth of lactobacilli (M ± m, n=3)**

Cultivation medium + selenium concentration (ppm)	Cultures of lactobacilli, Log CFU/cm^3	
	L. plantarum IMV B-7679	*L. casei* IMV B-7280
MRS control	7.65 ± 0.08	8.79 ± 0.22
MRS +1	7.36 ± 0.21	8.63 ± 0.18
MRS +3	6.36 ± 0.18	7.92 ± 0.12
MRS + 5	6.36 ± 0.06	6.41 ± 0.14
MRS +10	5.30 ± 0.07	5.79 ± 0.05*
MRS +30	5.17 ± 0.09*	4.41 ± 0.11*

* — statistically significant changes compared to control (cultivated cultures without sodium selenite addition), P ≥ 0.01.

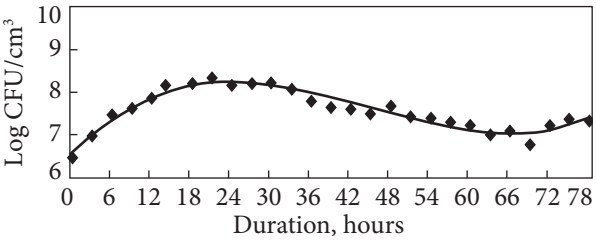

Fig. 1. *L. plantarum* IMV B-7679 strain growth curve

tions (Fig. 1). For the growth of *L. plantarum* IMV B-7679, there was used an MRS nutrient medium without introduction of inorganic selenium carriers. An important task was to check and compare the intensity of lactobacilli biomass on the standard nutrient medium of MRS under the conditions of normal and, in the case of introduction, different doses of sodium selenite. Under the condition of the norm, the lag-phase was not lasting because of physiologically young culture; the linear growth phase lasted 10—11 h. The phase of the slow growth of *L. plantarum* IMV B-7679 lasted 4—5 h. The stationary phase began at 15 h, and the culture growth curve came to the plateau. After 30 h, the death phase of *L. plantarum* IMV B-7679 began.

The impact of selenite ions on the growth of *L. plantarum* IMV B-7679 and *L. casei* IMV B-7280 was was studied in presence from 1 to 10 ppm sodium selenite. As a sowing material, there was used a crop from the exponential phase of growth on MRS nutrient medium. An important task of this work was to study the impact of sodium selenite by Se in its composition on the growth of *L. casei* IMV B-7280 and *L. plantarum* IMV B-7679. The research results have found that the dose of Se in sodium selenite impacts on the growth of lactobacilli, and sodium selenite inhibits the growth of bacteria starting from 24 h of observation. Further studies have revealed a gradual decrease in the concentration of bacteria by 48 h of observation. The change in the color of the environment under the influence of Na_2SeO_3 when cultivating lactobacilli was noted in the transition from the logarithmic phase of growth of crops to a stationary, which is consistent with other data [13].

The extension of the lag phase takes place under the conditions of enrichment of the cultural environment with sodium selenite by Se (3—5 ppm) in the case of *L. casei* IMV B-7280 or *L. plantarum* IMV B-7679 cultivation. It is known [13] that the enrichment with Na_2SeO_3 for *Azospirillum brasilense*, which is capable of restoring selenite to biogenic nanoselenium (Nano-Se), leads to the extension of the lag phase. The synthesis of Nano-Se *Bacillus subtilis* has been investigated [14]. The authors note that reduction of SeO_3^{2-} ions can begin during the transition from the logarithmic growth phase to the stationary one [13]. Under the influence of the investigated lactobacteria, the transformation of sodium selenite with the formation of biogenic Nano-Se was observed, which was accompanied by a decrease in the viability of bacteria. Note that the enrichment of the culture medium with Na_2SeO_3 was accompanied by a significant decrease in the biomass of *L. casei* IMV B-7280.

According to the obtained data, the optimal conditions for the growth of *L. plantarum* IMV B-7679 and *L. casei* IMV B-7280 in the presence of Na_2SeO_3 were determined,and the range of the most successful concentrations was established, which was from 3 to 5 ppm. Recovery of selenite ions by *L. casei* IMV B-7280 and *L. plantarum* IMV B-7679 cultures was determined by the pink-red coloration of the bacterial cultures (Fig. 2), which indicates the reduction of Se^{4+} and the presence of Se^0 in the bacterial cells and medium [15].

As shown for *L. plantarum* IMV B-7679, the reduction of SeO_3^{2-} ions begins in the middle and at the end of the exponential phase and is accompanied by the lengthening of the lag phase. The slowdown of bacterial growth was observed under the action of selenite ions at a Se concentration of 5 ppm. As a result, the cultivation of *L. plantarum* IMV B-7679 in the presence of sodium selenite was extended to 2 days.

Both studied strains of *L. casei* IMV B-7280 and *L. plantarum* IMV B-7679 had the ability to grow and resist the presence of 5 ppm Se in the culture medium. The strain *L. plantarum* IMV B-7679 showed the greatest resistance to selenite ions at a Se concentration of 5 ppm, demonstrating a decrease in cell viability by only 1.12 ± 0.05 Log CFU/cm^3 after 48 h of incubation. At the same time, the *L. casei* IMV B-7280 strain grown in the presence of Se 5 ppm demonstrated a decrease in cell viability by 2.23 ± 0.07 Log CFU/cm^3 after 48 h of incubation. Both probiotic strains showed the ability to restore selenite ions along with the accumulation of intracellular Nano-Se and the release of Nano-Se into the culture medium, which was accompanied by the coloring of bacterial cultures in red-brown shades (Figs. 2, 3).

It was shown that under normal conditions on the MRSA nutrient medium, cultures of *L. casei* IMV B-7280 and *L. plantarum* IMV B-7679 (after cultivation for 2 days at 37 °C) formed small shiny white colonies, round in shape with even edges, with a diameter from 1.5 mm to 3 mm. In the case of sowing aliquots containing sodium selenite, a decrease in the viability of lactobacilli cultures was observed depending on the content of Na_2SeO_3 in the culture medium.

Under normal conditions, cultivation of *L. casei* IMV B-7280 and *L. plantarum* IMV B-7679 for 48 h allowed us to obtain a biomass yield of 8.79 ± 0.22 and 7.65 ± 0.08 Log CFU/cm^3, respectively. The effect of sodium selenite on the culture growth depended on the dose of Se in sodium selenite. Thus, the enrichment of the nutrient medium with 3 ppm Se in the composition of sodium selenite reduced the growth of *L. casei* IMV B-7280 and *L. plantarum* IMV B-7679 cultures, respectively, to 7.92 ± 0.12 and 6.36 ± 0.18 Log CFU/cm^3 after cultivation for 48 h at 30 °C.

The ability of lactobacilli to recover selenite ions was investigated on solid nutrient media. If the *L. plantarum* IMV B-7679 strain was sown on the MRSA nutrient medium, into which a solution of Na_2SeO_3 was introduced at a concentration of 10 ppm by Se, then after 48 h of incubation, small round colonies with a faint pink color were detected. The obtained data indicate the ability of *L. plantarum* IMV B-7679 to reduce the colorless salt Na_2SeO_3 to elemental selenium (Se^0). The use of lower concentrations (1—3 ppm of Se in the form of Na_2SeO_3) did not affect the change in morphology and cultural properties of *L. plantarum* IMV B-7679. Under the influence of a concentration of 5 ppm Se in the form of Na_2SeO_3, a tendency to decrease the viability of this strain of lactobacilli was revealed. The ability of *L. casei* IMV B-7280 and *L. plantarum* IMV B-7679 cultures to grow on MRSA

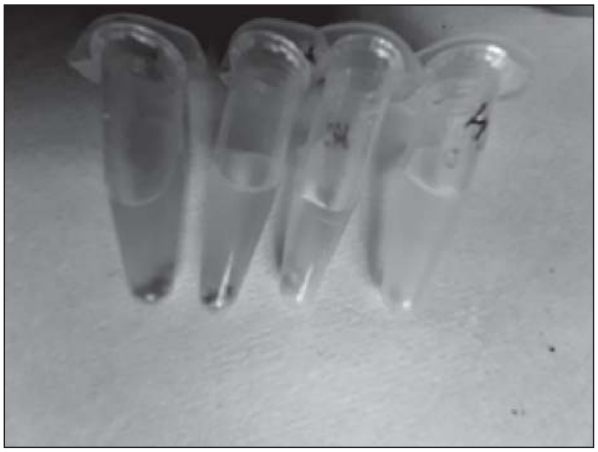

Fig. 2. Recovery of selenite ions by culture *L. casei* IMV B-7280 with enrichment of MRS (Na_2SeO_3)

Fig. 3. Color change of the culture medium of *L. plantarum* IMV B-7679 under conditions of using different concentrations of sodium selenite

nutrient medium in the presence of 3 ppm Se in the form of Na_2SeO_3 was shown. Higher tolerance to sodium selenite was found for culture of *L. plantarum* IMV B-7679. Thus, increasing the concentration to 30 ppm of Se in the form of Na_2SeO_3 led to a decrease in the viability of only the culture of *L. casei* IMV B-7280. That is, the studied lactobacilli showed different ability to grow in the presence of selenite ions.

The effect of selenite ions on *L. casei* IMV B-7280 and determination of the ability of the culture to form biogenic Nano-Se. The ability of the *L. casei* IMV B-7280 strain to transform sodium selenite with the formation of biogenic Nano-Se was investigated. For this, the sensitivity of this LAB strain to 1 or 5 ppm Se in the form of sodium selenite was tested. The strain *L. casei* IMV B-7280 was cultivated aerobically for 48 h in MRS nutrient medium enriched with 5 ppm Se in the form of Na_2SeO_3. Growth inhibition of *L. casei* IMV B-7280 was determined by plating aliquots on MRS nutrient medium. Under the influence of the studied concentrations of SeO_3^{2-} ions, growth inhibition of *L. casei* IMV B-7280 culture was observed.

When growing *L. casei* IMV B-7280 in the presence of selenium oxyanions, a change in the color of the bacterial culture medium to different shades of red occurred, which is the first sign of the transformation of Na_2SeO_3 into elemental Se^0, which was named biogenic selenium (Nano-Se). Thus, the *L. casei* IMV B-7280 strain was cultivated in MRS nutrient medium containing selenium oxyanions (SeO_3^{2-}) under optimized growth conditions. The recovery of selenite ions was determined by the color of bacterial cultures in shades of red, which indicates the presence of Se^0 in the medium [16].

At the beginning of the cultivation process, the *L. casei* IMV B-7280 strain in the presence of SeO_3^{2-} and in its absence (control) had the same color. On the second day of cultivation of *L. casei* IMV B-7280 in the MRS nutrient medium, which contained SeO_3^{2-}, a change in the color of the medium was observed compared to the control. Aliquots were taken from the culture medium with subsequent sedimentation of the bacterial cell suspension to determine the ability of *L. casei* IMV B-7280 to transform selenium oxyanions and form biogenic Nano-Se.

A suspension of bacterial cells with a volume of 1.5 cm^3 was precipitated in a microcentrifuge. The precipitate was resuspended in 1 cm^3 of 0.15 M NaCl solution. There was revealed a change in the color of the sediment of bacterial cells in the case of cultivation with SeO_3^{2-} to a pink-red color, which is a characteristic feature of bioreduction from Se^{6+} to Se^0. According to Xia et al. [15], the red color of the culture medium is due to the formation of non-toxic elemental selenium due to the reduction of Se^{6+} and the metabolism of high concentrations of selenium by lactobacilli (>4mg/dm^3) [17].

It was shown that strain *L. casei* IMV B-7280 is able to accumulate biomass in the presence of 5 ppm Se in the form of Na_2SeO_3 in the pH range from 6.0 to 8.0 at 30 °C under aerobic conditions. In the stationary phase in the absence of selenite (control), the amount of this strain reached $6.2 \pm 0.1 \cdot 10^8$ CFU/cm^3 at 30 °C under aerobic conditions. The introduction of 5 ppm Se^{6+} was accompanied by a decrease in the viability of the *L. casei* IMV B-7280 strain by 2 Log CFU/cm^3 and reached $2.6 \cdot 10^6$ CFU/cm^3 after 48 h of incubation at 30 °C in aerobic conditions.

The reduction of SeO_3^{2-} to Se^0 was determined by changing the color of the medium to different shades of red. This color can be caused by the products of oxidation-reduction processes that lead to the formation of elemental Se^0 nanoparticles of biogenic origin. The formation of Nano-Se was observed using Transmission Electron Microscopy (TEM).

Visualization of synthesized biogenic Nano-Se particles during the growth of *L. casei* IMV B-7280 in the presence of SeO_3^{2-}. Electronograms of the *L. casei* IMV B-7280 strain (performed on a JEM-1400 electron micro-

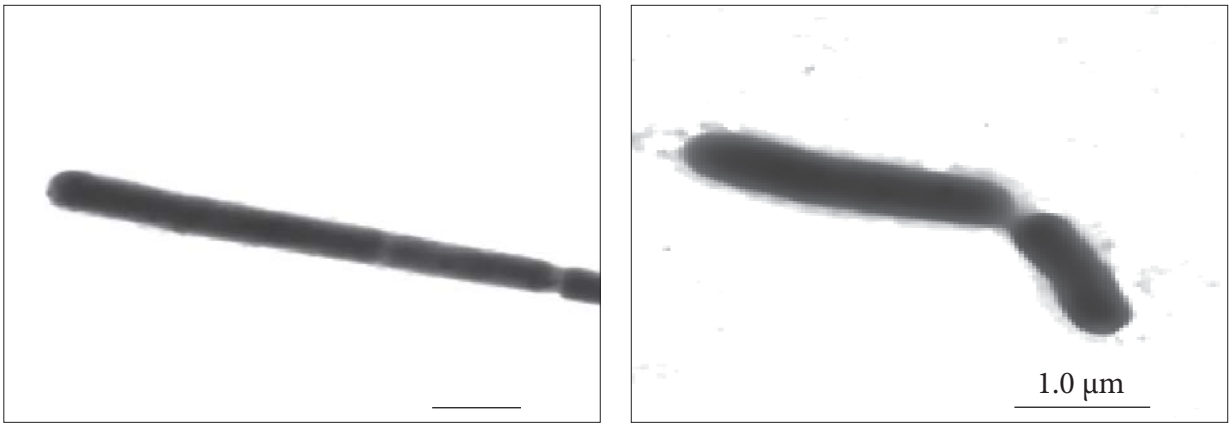

Fig. 4. Transmission electron microscopy (TEM) of *L. casei* IMV B-7280 (control) (bar — 1 μm)

scope) are presented in Fig. 4. Under normal conditions (without adding sodium selenite to the nutrient medium), strain *L. casei* IMV B-7280 was a stationary, gram-positive rod. The cells were 0.7—1.1×2.0—4.0 μm in size and did not form spores.

Cultivation of *L. casei* IMV B-7280 in the presence of 5 ppm Se^{4+} in the form of Na_2SeO_3 in MRS medium for 24 h was accompanied by significant ultrastructural changes in bacterial cells with their partial destruction and the formation of electron-dense Nano-Se particles inside bacterial cells (Fig. 5).

TEM methods allowed us to visualize the synthesized biogenic Nano-Se particles and determine their size. Measurements were carried out using a JEOL JEM 1400 device operating at an accelerating voltage of 80 kV.

To carry out TEM, aliquots of the suspension of bacterial cells, selected in the stationary phase of growth, with a volume of 1.5 cm^3 were precipitated in a microcentrifuge. The sediment was resuspended in 1 cm^3 of 0.15 M NaCl solution. Centrifugation of the culture for sedimentation of *L. casei* IMV B-7280 cells made it possible to remove the supernatant and wash the culture. TEM analysis revealed electron-dense spheres of different sizes: small (30—50 nm) and large ones with a diameter of approximately 150—250 nm. Large and small nanoparticles had a rounded shape, and some Nano-Se particles had a non-uniform surrounding.

Thus, under the influence of 5 ppm Se^{4+} for 24 h, that is, in the stationary phase of culture growth, a change in the morphology of *L. casei* IMV B-7280 cells was observed according to TEM data. Analysis of high-resolution TEM results (Fig. 6) revealed the presence of electron-dense Nano-Se particles inside bacterial cells and individual extracellular Nano-Se after 48 h of incubation of *L. casei* IMV B-7280, when the nutrient medium was enriched with sodium selenite 0.005 mg/cm^3, which is equal to 5 ppm Se^{4+}. Electron-dense particles in the cytoplasm of *L. casei* IMV B-7280 cells were different in size.

Discussion. The accumulation of Nano-Se by the culture of *L. casei* IMV B-7280 occurred intracellularly, with the subsequent release of selenium nanoparticles. The presence of a pink-red color and confirmation of the formation of Nano-Se made it possible to confirm the formation of Nano-Se using TEM and indicated the formation of amorphous red selenium [18, 19].

The formation of round electron-dense granules ranging in size from 30 nm to 250 nm was observed using TEM. Partial destruction of *L. casei* IMV B-7280 cells under the influence of selenium oxoanions was revealed, which was accompanied by the release of electron-dense Nano-Se particles synthesized by the culture.

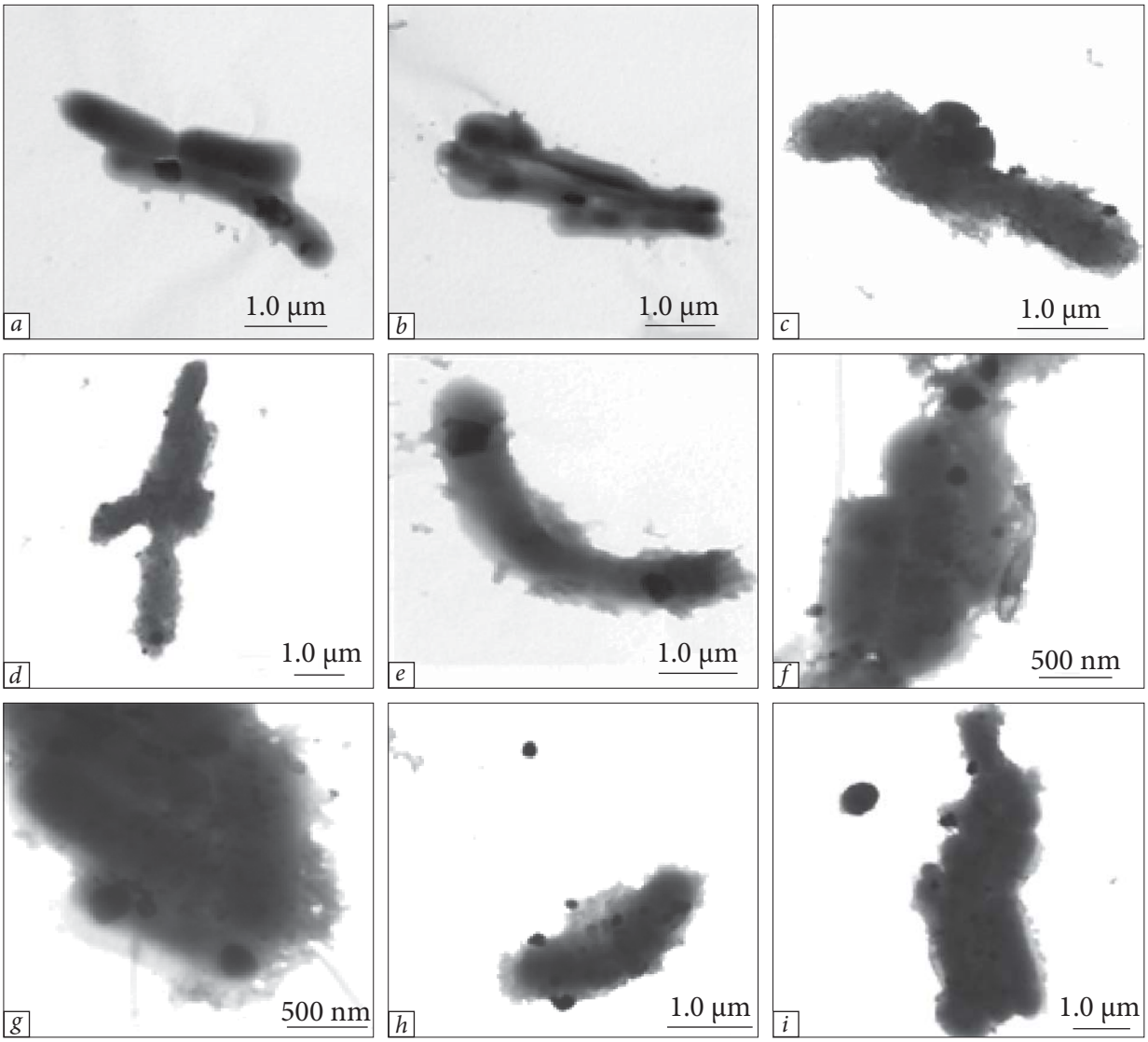

Fig. 5. TEM images of typical samples of *L. casei* culture IMV B-7280, which is capable of forming Nano-Se during growth on MRS nutrient medium in the presence of 5 ppm Se^{4+}: a, b, c, d (bar — 1 μm); e, f, g (bar — 500 nm) — electron-dense Nano-Se particles in the cytoplasm of *L. casei* IMV B-7280 cells; h, j — release of electron-dense Nano-Se lactomicroselenium particles into the culture medium; c —visualization of Nano-Se lactomicroselenium partially covered with the surrounding membrane

The synthesized Nano-Se particles were observed in the internal compartments of bacteria. *L. casei* IMV B-7280 cells had a disturbed structure. Also, With using TEM, the release or secretion of Nano-Se from damaged cells was revealed. It should be noted that the synthesized nanoparticles had different sizes from 50 nm to 250 nm, that is, they had variations in size, and part of them were more than 100 nm. Such Nano-Se particles were named Lactomicroselenium particles or lactomicroSel [20]. The increased size of lactomicroSel® above 100 nm is due to the coating of nanoparticles with a membrane provided by the bacterial cell. Note that *L. casei* showed the

ability to form Lactomicrosel® 100—200 nm in size, while *Bifidobacter* sp. — 400—500 nm [21].

As known, the biological activity of nanocomposites synthesized by lactobacilli depends on their encapsulation and coating with exopolysaccharides. The example of silver nanoparticles synthesized by lactobacilli shows that their antibacterial activity depends on the composition of exopolysaccharides of lactobacilli, which are involved in the synthesis of nanoparticles [22]. TEM analysis (Fig. 6) revealed the presence of extracellular electron-dense particles in the case of enrichment of the culture medium with 5 ppm Se^{4+} and aerobic cultivation for 24 h. The study with high-resolution TEM revealed rounded Nano-Se deposits of various sizes in the internal compartments of *L. casei* IMV B-7280 cells. The release of nanoparticles was accompanied by damage to *L. casei* IMV B-7280 cells.

Biogenic Nano-Se synthesized by *L. casei* IMV B-7280 culture accumulated in the bacterial cell during the exponential phase of growth, as determined by the change in the color of the culture medium and by the colored sediment of the bacterial cells. The production of Nano-Se was accompanied by the destruction of bacterial cells, which was proven by TEM and control over the viability of the culture under the conditions of using sodium selenite. The culture of *L. casei* IMV B-7280 when cultivated with sodium selenite produces nanoparticles from 30 nm to 250 nm. The reduction of sodium selenite to elemental selenium is a detoxification process. The synthesis of heterogeneous selenium particles by the culture *L. casei* IMV B-7280 is accompanied by the release of lactomicroSel selenium nanoparticles, the size of which often exceeds 100 nm. It should be noted that the multifaceted synthesis of nanoparticles was accompanied by the destruction of the culture. The formation of smaller granules by the culture of *L. casei* IMV B-7280 has a negative effect on bacterial cells. Since individual cells of *L. casei* IMV B-7280 are capable of synthesizing a significant number of small-sized nanoparticles, which are destroyed as a result of synthesis, the synthesis of larger lactomicrocell particles by L. casei IMV B-7280 culture has a less destructive effect on the cell structure compared to small Nano-Se. As it has been reported, smaller particles of lactomicrosela can be more toxic to the producing bacteria themselves [23].

It is known that the bacteria reduce SeO_3^{2-} to insoluble elemental selenium (Se^0) with the participation of the enzyme selenate reductase, however, the ability to form biogenic Nano-Se particles is a strain characteristic of the culture. Thus, lactobacilli have oxidoreductase systems for regulating the concentration of metals and metalloids [24]. Penetration of metals into the bacterial cell occurs with the help of the membrane system of electron transport and reducing enzymes.

Biogenic Nano-Se particles are produced by the culture of *L. casei* IMV B-7280 in the middle and at the end of the exponential phase and are secreted into the environment in the stationary phase [25, 26]. It was established [27] that *L. casei* 393 accumulated biogenic Nano-Se granules intracellularly, and the particle size was 50—80 nm. However, previous studies suggest that the size of nanoparticles produced by *L. casei* is 150—400 nm, while *L. acidophilus* produces lactomicroselenium particles of 200—350 nm. Lactobacteria are able to reduce Se^{4+} to Nano-Se in aerobic and/or anaerobic conditions. It was previously shown that *L. casei* 393 is capable of efficient transformation of sodium selenite into Nano-Se under anaerobic conditions [20].

We established the ability of *L. casei* IMV B-7280 to reduce selenite under aerobic conditions with the formation of Nano-Se particles of different sizes. The formation of small Nano-Se is accompanied by a violation of the integrity of the bacterial cell and a decrease in its viability. Along with the formation of small Nano-Se nanoparticles, larger Nano-Se lactomicroselenium granules with a size of 150—250 nm are formed. The

formation of Nano-Se lactomicroselenium leads to a partial disruption of the bacterial cell structure. The formed Nano-Se particles are released by the cells into the culture medium, which may be one of the mechanisms of Se detoxification by bacteria [28]. The TEM data established that the reduction of sodium selenite by the *L. casei* IMV B-7280 culture is accompanied by the formation of biogenic Nano-Se and a violation of the integrity and change of cell morphology. It is shown that the reduction of sodium selenite occurs intracellularly with the subsequent release of nanoparticles from bacterial cells. The mechanism of selenite recovery by *L. casei* IMV B-7280 is not yet sufficiently studied, but it is known that it includes at least two stages: transport of SeO_3^{2-} into the cell and intracellular redox processes.

Conclusions. In the presented study, the probiotic strains of lactobacilli *L. plantarum* IMV B-7679 and *L. casei* IMV B-7280 were tested for the synthesis of selenium nanoparticles by the processes of bioreduction of selenite ions, the possibility of their effective use in the green synthesis of selenium nanoparticles, and the prospects for practical application. It was established that the studied probiotic strains can restore selenite ions with the accumulation of intracellular Nano-Se and the release of Nano-Se into the culture medium. Based on the obtained data, optimal conditions for the growth of *L. plantarum* IMV B-7679 and *L. casei* IMV B-7280 in the presence of Na_2SeO_3 were determined, and the range of its most successful concentrations was determined. The research results prove that the ability to effectively form biogenic Nano-Se is a strain characteristic of the culture, which should be taken into account in the case of monitoring a promising probiotic strain of lactobacilli for the transformation of selenite to nanoselenium Nano-Se in order to create feed additives for animals containing an effective and safe form of selenium.

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БІОНАНОТЕХНОЛОГІЯ ВІДНОВЛЕННЯ СЕЛЕНІТ-ІОНІВ У НАНОСЕЛЕН ПРОБІОТИЧНИМИ ШТАМАМИ ЛАКТОБАКТЕРІЙ ТА ТОЛЕРАНТНІСТЬ ЛАКТОБАКТЕРІЙ ДО СЕЛЕНІТУ НАТРІЮ

Зелений синтез наночастинок (НЧ) з використанням живих клітин є перспективним і новим інструментом у біонанотехнологіях. Для синтезу НЧ використовують хімічні та фізичні методи, однак перевага віддається біологічним методам з огляду на їхні екологічні, чисті, безпечні, рентабельні, прості та ефективні джерела для високої продуктивності та чистоти. **Мета.** Дослідити процеси біовідновлення селеніт-іонів у наноселен пробіотичними штамами лактобактерій *Lactobacillus plantarum* IMB B-7679 і L. casei IMB B-7280. **Методи.** Культивування лактобактерій *L. plantarum* IMB B-7679 і *L. casei* IMB B-7280 проводили у флаконах (500 см^3) на ротаційному шейкері (220 об/хв) за температури 30 °C впродовж двох діб культивування в поживному середовищі MRS Broth. В середовище додатково вносили селеніт натрію в різних концентраціях від 1 до 30 ppm за Se. Кількість життєздатних клітин бактерій в 1 см^3 суспензії визначали методом граничних розведень у разі висіву аліквот на поживне середовище, яке містило 0,2 % агар-агару. Культури *L. plantarum* IMB B-7679 або *L. casei* IMB B-7280 вирощували в рідкому середовищі MRS Broth в присутності або відсутності Na2SeO3. Концентрація селеніту натрію складала від 1 до 30 ppm за Se. Визначення кількості мікроорганізмів проводили шляхом посіву (0,1 см^3 суспензії) на щільні середовища на чашки з MRS агаром, посівна доза складала 107 кл/чашку Петрі. Толерантність лактобактерій до впливу селеніт-іонів визначали за зниженням кількості КУО при висіві аліквот, відібраних від зразків культури, що росла у присутності селеніту або за його відсутності. Результати дослідів представляли у КУО та переводили у Log КУО/см^3. Характеристики Nano-Se вивчали за допомогою трансмісійної електронної мікроскопії (ТЕМ). **Результати.** З'ясовано, що після 48 год інкубації на середовищі MRS з додаванням селеніту натрію від 1 до 30 ppm найбільш стійкою виявилась культура *L. plantarum* IMB B-7679. Так, збагачення культурального середовища селеном 30 ppm у складі Na_2SeO_3 приводило до зниження кількості L. plantarum IMB B-7679 — до 5.17 ± 0.09 Log КУО/см^3 проти 4.41 ± 0.11 Log КУО/см^3 для *L. casei* IMB B-7280. Використання нижчих концентрацій (1—3 ppm Se у складі Na_2SeO_3) не вливало на зміну морфології та культуральні властивості *L. plantarum* IMB B-7679. Показано здатність культур *L. casei* IMB B-7280 та *L. plantarum* IMB B-7679 до росту на поживному середовищі MRSA в присутності 3 ppm Se у вигляді Na_2SeO_3. Вищу толерантність до селеніту натрію виявила культура *L. plantarum* IMB B-7679. Так, підвищення концентрації до 30 ppm Se у складі Na_2SeO_3 призводило до зниження життєздатності лише культури *L. casei* IMB B-7280. Тобто, досліджувані лактобактерії виявили різну здатність до росту в присутності селеніт-іонів. За допомогою ТЕМ спостерігали утворення круглих електронно-щільних гранул розміром від 30 нм до 250 нм. Обидва пробіотичні штами виявили властивість до відновлення селеніт-іонів з накопиченням внутрішньоклітинно Nano-Se та вивільненням його в культуральне середовище, що супроводжувалось забарвленням бактеріальних культур у відтінки червоно-коричневого кольору. Виявлено часткову деструкцію клітин *L. casei* IMB B-7280 за впливу оксоаніонів селену, що супроводжувалось вивільненням синтезованих культурою електронно-щільних частинок Nano-Se. **Висновки.** В результаті проведеної роботи встановлено оптимальні умови для збільшення біомаси *L. plantarum* IMB B-7679 та *L. casei* IMB B-7280 в присутності Na_2SeO_3 та доведено, що лактобактерії мають різну здатність до росту в присутності селеніт-іонів. Отримані дані свідчать, що досліджувані пробіотичні штами мають здатність до відновлення селеніт-іонів з накопиченням внутрішньоклітинно Nano-Se та вивільненням його в культуральне середовище.

Ключові слова: *зелений синтез, селеніт натрію, наночастинки селену, пробіотичні штами, L. plantarum IMB B-7679, L. casei IMB B-7280, ТЕМ.*