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STUDY OF GENUS *BACILLUS* (*B. CLAUSII*) PROBIOTIC BACTERIA REGARDING THE BIOGENIC EXTRACELLULAR SYNTHESIS OF SELENIUM NANOPARTICLES

The biogenic method of nanoparticle synthesis with the participation of microorganisms that are capable of producing nanomaterials of different shapes, sizes, and chemical compositions is a promising innovative direction of nanotechnology. Bacteria are chosen for the production of nanoparticles due to their rapid reproduction, ease of cultivation, low energy requirements, and minimal costs. The complex synthetic mechanisms available to microorganisms allow them to use a large number of building blocks to construct new biosynthetic nanostructures that can accumulate in vesicles inside the cell or by extracellular synthesis. In the modern world, the so-called «green» technologies come to the fore, and the active studies of microorganisms with a high enzymatic potential, which can be used in nanobiotechnology and are promising for practical application, are being actively expanded. We have screened strains of Bacillus bacteria for their ability to reduce Se (IV) in the composition of sodium selenite to Se^0. The ***aim*** *of the research was to study the processes of biogenic synthesis of selenium nanoparticles by probiotic strains of Bacillus clausii and their prospects for practical application.* ***Methods.*** *Cultivation of B. clausii was carried out in vials (500 cm^3) on a rotary shaker (20 rpm) at of 30 °C for 3 days on a nutrient medium of MPB. Sodium selenite 0.0065 g/100 mL was additionally added to the medium. A visual assessment of the color change in the nutrient culture medium was carried out under the conditions of its enrichment with 30 ppm Se in the composition of sodium selenite. The characteristics of nano-Se were studied using transmission electron microscopy (TEM).* ***Results.*** *It was established that the addition of sodium selenite 0.0065 g/100 mL (30 ppm Se) within the composition of sodium selenite to the nutrient medium revealed the ability of B. clausii to reduce oxyanions Se (IV) into nanoparticles of elemental selenium Se^0 (appearance of orange color). Bacterial cells and biosynthesized selenium*

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nanoparticles were separated for further transmission microscopy. Synthesized Nano-Se nanocrystals were detected in TEM images. Nano-Se particle sizes determined from TEM images varied within 298±52 nm. Nanoparticles obtained by B. clausii formed conglomerates of nanocrystals; individual nanoparticles had a spherical shape. A change in the color of the environment under the influence of Na_2SeO_3 during the cultivation of B. clausii was noted when the growth phase of the cultures went from logarithmic to stationary mode. Research has established for the first time that B. clausii is capable of reducing selenite to elemental selenium, as evidenced by TEM data. ***Conclusions****. The obtained data indicate the ability of B. clausii to reduce sodium selenite with the formation of extracellular selenium nanoparticles (Nano-Se). The B. clausii-assisted transformation of sodium selenite with extracellular deposits of Nano-Se opens an accessible source of biogenic Nano-Se and the creation of selenium-containing probiotic preparations based on it.*

Keywords*: biogenic synthesis, sodium selenite, selenium nanoparticles, probiotic strains, Bacillus clausii, TEM, extracellular synthesis.*

Statement of the problem and analysis of the recent research. Biogenic nanotechnology is an interdisciplinary field that combines biology and materials science. Green synthesis of nanoparticles using environmentally friendly methods and materials has become an important area of research in recent years. Nanoparticles are becoming more and more popular in various industries due to their unique properties and application possibilities. However, conventional methods of nanoparticle synthesis have several limitations, including the use of hazardous chemicals, high energy requirements, and high costs (Tao, Huachen et al., 2021). Green nanoparticles, are produced using environmentally friendly methods and materials and have several advantages over conventional nanoparticles. One of the main advantages of green nanoparticles is their biocompatibility. They are often made using non-toxic, biodegradable, and renewable materials, making them safe for use in a variety of applications. Green nanoparticles also have improved properties compared to conventional nanoparticles, such as increased stability and bioactivity. Another advantage of green nanoparticles is their cost-effectiveness. Traditional synthetic methods of nanoparticle synthesis can be expensive, but ecological synthesis methods are often more affordable. In addition, green nanoparticles are environmentally sustainable and contribute to sustainable development by reducing the use of hazardous chemicals and promoting the use of renewable resources. The biogenic method of nanoparticle synthesis is a particularly promising approach to obtaining green nanoparticles (Tymoshok et al., 2019). This method uses natural biological systems such as plants, animals, fungi, and bacteria to produce nanoparticles, resulting in a more environmentally friendly and cost-effective process. For example, plant extracts contain phytochemicals necessary for the synthesis of nanoparticles and for enhancing their bioactivity.

Nanoforms of selenium have attracted considerable attention due to their high bioavailability and lower toxicity compared to inorganic and organic forms of Se (Bokulich et al., 2016; Stewart et al., 2002; Zhang et al., 2008; The target program of fundamental research of the NASU 0120U102297, OK: 0222U004405). In addition, inorganic compounds of selenium are more toxic than organic ones (Gordon et al., 2010).

The development of an environmentally friendly and inexpensive method of synthesis of nanoparticles is of crucial importance. There are numerous organisms capable of synthesizing nanoparticles and having the potential to use them. Significant applications of nanomaterials are usually size-dependent, so size-controlled synthesis of nanomaterials is highly desirable.

Microorganisms are also increasingly being used to produce nanoparticles. Despite the unique reaction mechanism of each biogenic material, they all function essentially the same, resulting in the production of the desired nanostructures. In addition, biogenic synthesis can

be used to create biomimetic materials, which have advanced biomedical applications. The use of biopolymers, plant extracts, and biomolecules in green synthesis is of increasing interest. These reagents are biocompatible and function in a variety of ways, such as blocking, reducing, and modulating agents. Thus, the biogenic method of nanoparticle synthesis is a promising approach for obtaining green nanoparticles. The use of environmentally friendly procedures, such as the use of biopolymers, plant extracts, and biomolecules, is of increasing interest to achieve this goal.

Microorganisms can produce nanomaterials of different shapes, sizes, and chemical compositions. The complex synthetic mechanisms available to microorganisms allow them to use a large number of building blocks to construct new biosynthetic nanostructures that can accumulate in vesicles inside the cell or be transported outside. In this way, possible toxicological effects can be avoided. This inherently complex synthesis of nanomaterials has been a source of biological inspiration and biomimicry for many years. In particular, bacteria were chosen for the production of nanoparticles due to their rapid reproduction, ease of cultivation, low energy requirements, and minimal costs (Antezana et al., 2022). There are reports on the biosynthesis and most important properties of NPs (gold, silver, copper, selenium) obtained by bacterial methods, and the suggested intracellular and extracellular mechanisms of their bacterial biosynthesis are also discussed.

Molecular mechanisms of the production of selenium nanoparticles aerobically from selenite with the participation of the bacterium *Pseudomonas putida* KT2440 were presented for the first time in research (Avendaño et al., 2023). The obtained results indicate that the reduction of selenite to nanoselenium occurs through an interconnected metabolic network, which includes central metabolic reactions, in particular 2-oxoglutarate/glutamate metabolism (controlled by genes sucA, D2HGDH and PP_3148), sulfur metabolism (regulated by genes *cysG, sqr p do2, sqrR, ssuEF*,and *sfnCE*), as well as response to oxidative stress (*Gqr, lsfA, ahpCF,* and *sadI* genes). Importantly, these steps are combined to produce glutathione. Mutations affecting sulfite reductase activity reduced the bacteria's ability to transform selenite into nanoselenium. Scientists isolate genes that were not linked to selenium metabolism in other bacteria and focus on genes of biotechnological interest (sqrR, pdo2, and sqr), whose inactivation results in the production of selenium nanoparticles at a higher rate than the original strain bacteria. This provides prospects for the development of biotechnological tools to increase the efficiency of bionanotechnology for the production of nanoselenium with the participation of bacteria. Transcriptome studies have proven the effect of selenite on increasing the expression of membrane-associated proteins, and these changes in the expression patterns of membrane proteins are explained by the fact that the restoration of selenite leads to the accumulation of extracellular selenium nanoparticles and causes the rearrangement of the membrane proteome.

Recently, the first attempts were made to synthesize selenium nanoparticles, which were obtained extracellularly using the probiotic bacteria *B. clausii*, and their antibacterial effectiveness against multiresistant bacteria was evaluated. Biosynthesized Nano-Se showed antibacterial activity against most multidrug-resistant (MDR) bacteria, such as *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Klebsiella pneumonia, Acinetobacter baumannii, Proteus mirabilis, Pseudomonas aeruginosa*, and *Shigella sonii* in comparison with the results of the antibiotic study of ceftriaxone, azithromycin, cefepime, amikacin, meropenem, oxacillin, gentamicin, and ciprofloxacin. The authors envisage the use of probiotics and Nano-Se to enhance or replace antibiotics (Al-Shemmary et al., 2022). Wide enzymatic activity of *B. clausii* and the presence of intracellular sulfite reductase and nitrate reductase enzymes were established (Beller et al.,

2013). The presence of NADPH-dependent enzymes, including nitrate reductase, which plays a key role in the formation of nanoparticles, was revealed in the structure of *B. clausii*. The ability of *B. clausii* bacteria cultured from probiotic Enterogermin (Sanofi India Ltd.) to the extracellular biosynthesis of silver nanoparticles due to the significant nitrate reductase activity has been investigated by (Nadaf et al., 2019).

Currently, *Bacillus* cultures *coagulans*, *subtilis*, *clausii*, *toyoi*, *lichemiformis*, *mesentericus*, and *polymyxa* are included in probiotic preparations.

Enzymes produced by *B. subtilis* and *B. licheniformis* contribute to the improvement of digestion and assimilation of food, as well as the cleansing of wounds and inflammatory foci from necrotized tissues. The ability of *B. subtilis* IMB B-7393 and IMB B-7392 strains to reduce Se (IV) in the form of sodium selenite to Se^0 (Vaidyanathan et al., 2010; Tymoshok et al., 2019) and to accumulate more than 1 mM of sodium selenite was revealed. Such reports served as a basis for further research on the ability of *B. clausii* to transform sodium selenite into an elemental Se state.

It was shown that for the culture of *B. clausii*, NADPH-dependent nitrate reductase is responsible for the biosynthesis of nanosilver. There is a report on the ability of *B. clausii* to biosynthesize gold nanoparticles (Beller et al., 2013; Shi et al., 2011).

By changing the color of the medium, the cultivation of *B. clausii* in the presence of sodium selenite 0.006 g/100 mL, i.e. 30 ppm Se, led to the appearance of an orange-brick color, which indicated the reduction of Se (IV) and the formation of Nano-Se.

All these studies have become the basis for studying the enzymatic potential of *B. clausii* for the synthesis of Nano-Se, since previously the culture of *B. clausii* was not used specifically for the biosynthesis of Nano-Se.

The **purpose** of the research was to study the processes of biogenic synthesis of selenium nanoparticles by probiotic strains of *B. clausii*.

Materials and Methods. Cultivation of *B. clausii* was carried out in vials (500 cm^3) on a rotary shaker (20 rpm) at a 30 °C for 3 days of cultivation on the MPB nutrient medium. Sodium selenite was additionally added to the medium in a dose of 0.0065 g/100 mL. A visual assessment of the color change in the nutrient culture medium was carried out under the conditions of its enrichment with 30 ppm Se in the composition of sodium selenite. Nano-Se characteristics were studied by transmission electron microscopy (TEM) using a JEM-1400 electron microscope (Japan).

Results. This research has established for the first time that *B. clausii* is capable of reducing selenite to elemental selenium, which is evidenced by the appearance of orange color in the medium (Fig. 1).

The addition of sodium selenite in the amount of 0.0065 g/100 mL (30 ppm Se) within the composition of sodium selenite to the nutrient medium revealed the ability of *B. clausii* 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 *B. clausii* was noted in the case of transition from the logarithmic phase of culture growth to the stationary one, which is consistent with the literature data (Wang et al., 2007). Further research on Nano-Se was carried out after the purification of the nanoforms by centrifugation.

To confirm the Nano-Se formation and clarify the morphology of nanoparticles, TEM analysis was performed. For the first time, based on the analysis of data on the involvement of the nitrate reductase enzyme in the synthesis of nanoparticles, we confirmed the formation of Nano-Se during the transformation of sodium selenite by the *B. clausii* culture (Fig. 2).

TEM was conducted to find out the microstructural features of the obtained samples.

TEM image analysis showed that the synthesized electron-dense Nano-Se particles existed extracellularly in the microenvironment of cells of the *B. clausii* strain in the form of nanoag-

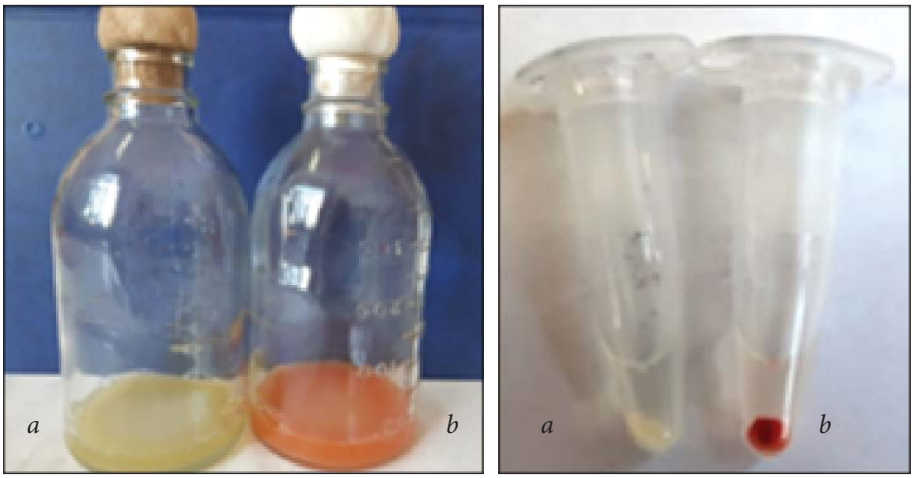

Fig. 1. The formation of nanoselenium particles under the conditions of 72-hour cultivation of *B. clausii* in a medium containing 30 ppm Se in the composition of sodium selenite (*1*) and purification of the obtained nanoforms by centrifugation (*2*): *a* — control; *b* — experiment

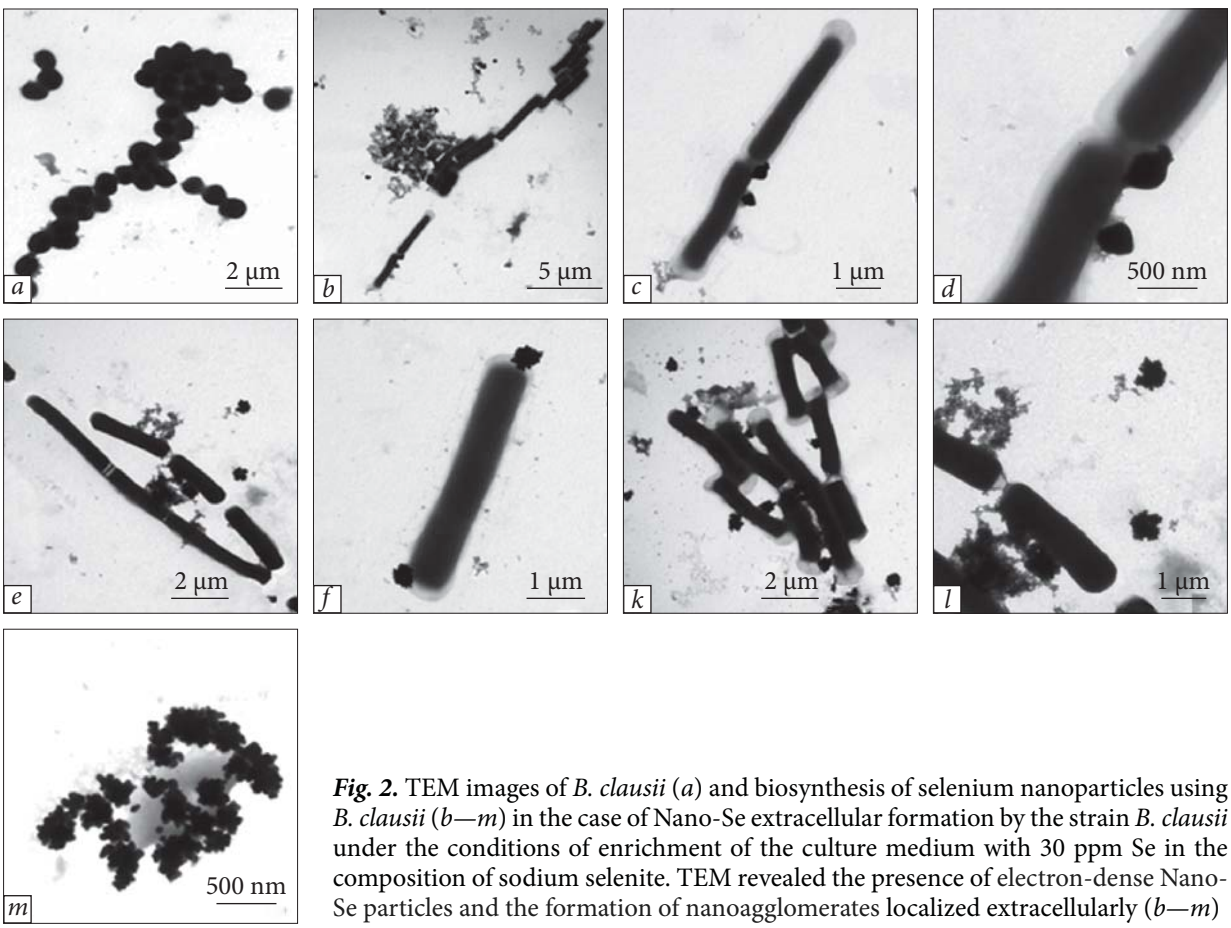

Fig. 2. TEM images of *B. clausii* (*a*) and biosynthesis of selenium nanoparticles using *B. clausii* (*b*—*m*) in the case of Nano-Se extracellular formation by the strain *B. clausii* under the conditions of enrichment of the culture medium with 30 ppm Se in the composition of sodium selenite. TEM revealed the presence of electron-dense Nano-Se particles and the formation of nanoagglomerates localized extracellularly (*b*—*m*)

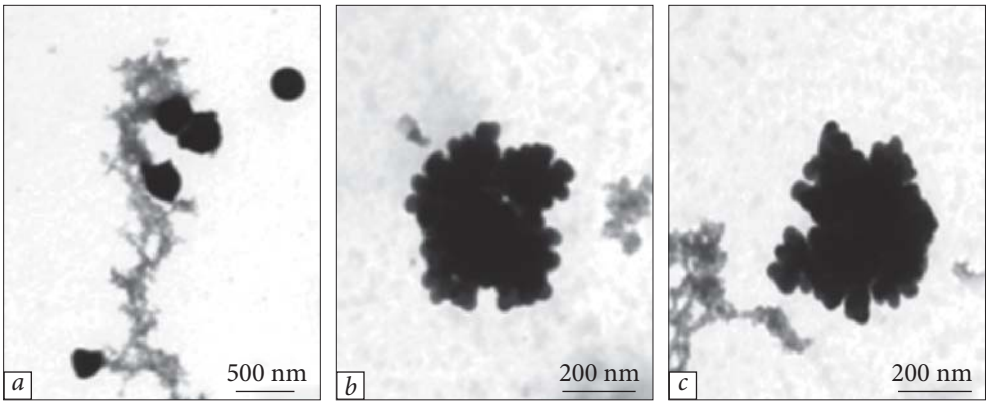

Fig. 3. TEM images of nanoparticles produced by *B. clausii* under the conditions of enrichment of the culture medium with 30 ppm Se in the composition of Na_2SeO_3

gregates. That is, when enriching the culture medium with 30 ppm Se (IV) and cultivating aerobically for 48 h at 30 °C, these extracellular electron-dense NPs are agglomerated.

The formation of sediment by *B. clausii* culture is shown for the first time. The transformation of *B. clausii* sodium selenite with extracellular deposits of nanoselenium opens an accessible source of biogenic Nano-Se and the creation of selenium-containing probiotic preparations based on it.

A separate part of the work was devoted to obtaining Nano-Se, i.e. separation of bacterial cells and formed nanoparticles for further transmission microscopy. Synthesized conglomerates of Nano-Se nanocrystals can be distinguished in TEM images (Fig. 3). Nano-Se particle sizes determined from TEM images vary within 298±52 nm.

Nanoparticles formed by *B. clausii* form conglomerates of nanocrystals. Some nanoparticles have a spherical shape. A change in the medium color under the influence of Na_2SeO_3 during the cultivation of *B. clausii* was noted during the transition from the logarithmic phase of culture growth to the stationary phase.

The reduction of Se in SeO_4^{2-} to SeO_3^{2-} is mainly catalyzed by soluble or membrane-bound selenate reductase (Ser), which combines three subunits that have molybdenum as a cofactor located in the periplasm or on the cytoplasmic membrane.

Discussion. Due to the presence of NADPH-dependent nitrate reductase enzymes, which are involved in the biosynthesis of nanoparticles, *B. clausii* is able to form silver and gold NPs. NADH-dependent nitrate reductase, which acts as an electron shuttle, accepts electrons from nitrate and transfers them to the metal ion to form nanoparticles, as illustrated for *F. oxysporum*, *P. aeruginosa*, and other microorganisms (Kalimuthu et al., 2008; Mikhailova et al., 2020; Mohd Yusof et al., 2019).

Involvement of transmembrane respiratory nitrate reductase (Nar) (Downey, 1966) and periplasmic nitrate reductase (Nap) (Tao, Huachen et al., 2021) are very important in the biosynthesis of NPs (Gupta, 1997). It should be noted that the active center of these enzymes is molybdenum. In particular, a membrane-bound nitrate reductase was found in the bacterium *Bacillus licheniformis*, which is involved in the synthesis of silver nanocrystals (Kumar et al., 2007). At the same time, the recovery of metals or metalloids occurs in response to the introduction of the corresponding ions and is caused by the stress reaction of the microorganism to a change in the environment (Mukherjee et al., 2018).

The research results show that *B. clausii*, which was cultivated from the probiotic *Enterogermin* (Sanofi India Ltd.), is appropriate to use for obtaining probiotic selenium-containing preparations, since *B. clausii* is capable of transforming sodium selenite into an elemental state.

In the modern world, the so-called «green» technologies take first place, and the active studies of microorganisms that have a high enzymatic potential to be used in nanobiotechnology and are promising for further research and practical application are being conducted. We screened strains of *Bacillus* bacteria for their ability to reduce Se (IV) in the form of sodium selenite to Se^0, which required additional research, including by TEM.

Our research showed for the first time the ability of *B. clausii* to reduce sodium selenite with the formation of Nano-Se. However, the confirmation of the probability of involvement of the selenate reductase enzyme of the culture of this strain in the recovery of Se (IV) remains unclear. For *Thauera selenatis*, the ability of periplasmic nitrate reductase to catalyze the reduction of selenite to elemental selenium was shown. In addition, the ability of *B. clausii* to form silver nanoparticles with the help of nitrate reductase was proven. In addition, anaerobic respiration of *Bacillus stearothermophilus* has been shown even earlier due to inducible nitrate reductase (Fajt et al., 2009).

In the applied aspect, it should be noted that the enzymes nitrate reductase (NaR) and nitrite reductase (NiR) are able to ensure the extracellular synthesis of nanoparticles (Bharti et al., 2020). Nitrate reductase converts nitrate to nitrite. The enzyme complex of the denitrifying bacteria *T. pantotropha* has two types of nitrate reductase enzymes, i.e., the membrane-bound NaR enzyme, which is active only under anaerobic conditions, the periplasmic NaR enzyme (active under aerobic conditions), and the nitrite reductase (NiR) enzyme, which reduces nitrite to N_2 (Hallol et al., 2013).

Some authors suggest using *Bacillus licheniformis* culture and its enzymatic potential, namely nitrate reductase, for the reduction of Ag^+ and the production of silver nanoparticles. In particular, the widespread mechanism of silver biosynthesis with the participation of bacteria is the action of the enzyme nitrate reductase. For *Bacillus licheniformis*, during bacterial reduction, alpha-nicotinamide-adenine-dinucleotide phosphate reduced form of NADPH-dependent nitrate reductase is involved. In the case of reduction, the nitrate turns into nitrite, and the electron is transferred to the silver ion; therefore, the silver ion (Ag^+) is reduced to Ag^0 (Jeevan et al., 2012; Vogel et al., 2018).

Conclusions. The obtained data indicate the ability of *B. clausii* to reduce sodium selenite with the formation of extracellular selenium nanoparticles (Nano-Se). For the *B. clausii* culture, the involvement of a nitrate reductase in the biosynthesis of silver nanoparticles has been proven; in our research, the ability of *Bacillus clausii* to transform selenite into extracellular Nano-Se was established for the first time (the target program of fundamental research NASU 0120U102297, OK: 0222U004405). However, further analysis and determination of the profile of the reductase enzyme and the understanding of the enzyme activity at the proteomic level will help to develop more effective strategies and practices for obtaining nanoparticles of extracellular localization. Therefore, the following experiments are aimed at comparative studies of the ability to transform sodium selenite and the formation of extracellular Nano-Se by different probiotic strains of the genus *Bacillus*.

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Conflicts of interest. The authors declare no potential conflict of interest concerning the article.

REFERENCES

Al-Shemmary, A. J., Malallah, H. A., Al-Mashhadi, A. R., Jaber, A. H., & Shaker, Z. B. (2022). Biosynthesis of selenium nanoparticles using probiotic *Bacillus clausii* and their antibacterial efficacy against multidrug-resistant bacteria (MDR). *Journal of Pharmaceutical Negative Results,* 1011—1019.

Antezana, P. E., Municoy, S., & Desimone, M. F. (2022). Building nanomaterials with microbial factories. *Biogenic Sustainable Nanotechnology*, 1—39.

Avendaño, R., Muñoz-Montero, S., Rojas-Gätjens, D., Fuentes-Schweizer, P., Vieto, S., Montenegro, R., & Jiménez, J. I. (2023). Production of selenium nanoparticles occurs through an interconnected pathway of Sulphur metabolism and oxidative stress response in *Pseudomonas putida* KT2440. *Microbial Biotechnology, 16*(5), 931—946.

Beller, H. R., Han, R., Karaoz, U., Lim, H., & Brodie, E. L. (2013). Genomic and physiological characterization of the chromate-reducing, aquifer-derived firmicute *Pelosinus* sp. strain HCF1. *Applied and environmental microbiology, 79*(1), 63—73.

Bharti, S., Mukherji, S., & Mukherji, S. (2020). Extracellular synthesis of silver nanoparticles by *Thiosphaera pantotropha* and evaluation of their antibacterial and cytotoxic effects. *3 Biotech, 10*, 1—12.

Bokulich, N. A., Lewis, Z. T., Boundy-Mills, K., & Mills, D. A. (2016). A new perspective on microbial landscapes within food production. *Curr Opin Biotechnol, 37*, 182—189.

Deepak, V., Kalishwaralal, K., Pandian, S. R. K., & Gurunathan, S. (2011). An insight into the bacterial biogenesis of silver nanoparticles, industrial production and scale-up. *Metal nanoparticles in microbiology*, 17—35.

Downey, R. J. (1966). Nitrate reductase and respiratory adaptation in *Bacillus stearothermophilus*. *Journal of Bacteriology, 91*(2), 634—641.

Fajt, Z., Drabek, J., Steinhauser, L., & Svoboda, M. (2009). The significance of pork as a source of dietary selenium—An evaluation of the situation in the Czech Republic. *Neuroendocrinology Letters, 30*(1), 17.

Gordon, O., Vig Slenters, T., Brunetto, P. S., Villaruz, A. E., Sturdevant, D. E., Otto, M., & Fromm, K. M. (2010). Silver coordination polymers for prevention of implant infection: thiol interaction, impact on respiratory chain enzymes, and hydroxyl radical induction. *Antimicrobial agents and chemotherapy, 54*(10), 4208—4218.

Gupta, A. B. (1997). *Thiosphaera pantotropha*: a sulphur bacterium capable of simultaneous heterotrophic nitrification and aerobic denitrification. *Enzyme and Microbial Technology, 21*(8), 589—595.

Hallol, M. (2013). Studies on Bacterial Synthesis of Silver Nanoparticles Using Gamma Radiation and Their Activity against Some Pathogenic Microbes. Applied Life Science, International Atomic Energy Agency, 18.

Jeevan, P., Ramiya, K., & Rena, A. E. (2012). Extracellular biosynthesis of silver nanoparticles by *Pseudomonas aeruginosa* culture supernatant. *Indian Journal of Biotechnology, 11*, 72—76.

Kalimuthu, K., Babu, R. S., Venkataraman, D., Bilal, M., & Gurunathan, S. (2008). Biosynthesis of silver nanocrystals by *Bacillus licheniformis*. *Colloids and surfaces B: Biointerfaces, 65*(1), 150—153.

Kumar, S. A., Abyaneh, M. K., Gosavi, S. W., Kulkarni, S. K., Pasricha, R., Ahmad, A., & Khan, M. I. (2007). Nitrate reductase-mediated synthesis of silver nanoparticles from $AgNO_3$. *Biotechnology Letters, 29*(3), 439—445.

Mikhailova, E. O. (2020). Silver nanoparticles: mechanism of action and probable bio-application. *Journal of functional biomaterials, 11*(4), 84.

Mohd Yusof, H., Mohamad, R., Zaidan, U. H., & Abdul Rahman, N. A. (2019). Microbial synthesis of zinc oxide nanoparticles and their potential application as an antimicrobial agent and a feed supplement in animal industry: a review. *Journal of animal science and biotechnology, 10*, 1—22.

Mukherjee, K., Gupta, R., Kumar, G., Kumari, S., Biswas, S., & Padmanabhan, P. (2018). Synthesis of silver nanoparticles by *B. clausii* and computational profiling of nitrate reductase enzyme involved in production. *Journal of Genetic Engineering and Biotechnology, 16*(2), 527—536.

Nadaf, N. Y, & Kanase, S. S. (2019). Biosynthesis of gold nanoparticles by *Bacillus marisflavi* and its potential in catalytic dye degradation. *Arabian Journal of Chemistry, 12*(8), 4806—4814.

Shi, L., Xun, W., Yue, W., Zhang, C., Ren, Y., Shi, L., & Lei, F. (2011). Effect of sodium selenite, Se-yeast and nano-elemental selenium on growth performance, Se concentration and antioxidant status in growing male goats. *Small Ruminant Research, 96*(1), 49—52.

Stewart, V., Lu, Y., & Darwin, A. J. (2002). Periplasmic nitrate reductase (NapABC enzyme) supports anaerobic respiration by *Escherichia coli* K-12. *Journal of Bacteriology, 184*(5), 1314—1323.

Tao, Huachen, et al. (2021). «Nanoparticle synthesis assisted by machine learning.» *Nature reviews materials* 6.8, 701—716.

The target program of fundamental research of the National Academy of Sciences of Ukraine «Promising fundamental research and innovative development of nanomaterials and nanotechnologies for the needs of industry, health care and agriculture» for 2020—2024»—»Development of scientific foundations of biotechnology for obtaining safe, selenium-containing probiotic preparations for increasing productivity and preservation of poultry stock» (state registration number 0120U102297, OK: 0222U004405).

Tymoshok, N. O., Demchenko, O. A., Bityutskyy, V. S., Tsekhmistrenko, I., Kharchuk, M. S., & Tsekhmistrenko, O. S. (2023). Bionanotechnology of Selenite Ions Recovery into Nanoselenium by Probiotic Strains of *Lactobacteria* and Tolerance of Lactobacteria to Sodium Selenite. *Mikrobiol Z, 85*(4), 9—20.

Tymoshok, N. O., Kharchuk, M. S., Kaplunenko, V. G., Bityutskyy, V. S., Tsekhmistrenko, S. I., Tsekhmistrenko, O. S., & Melnichenko, O. M. (2019). Evaluation of effects of selenium nanoparticles on *Bacillus subtilis*. *Regulatory Mechanisms in Biosystems, 10*(4), 544—552.

Vaidyanathan, R., Gopalram, S., Kalishwaralal, K., Deepak, V., Pandian, S. R. K., & Gurunathan, S. (2010). Enhanced silver nanoparticle synthesis by optimization of nitrate reductase activity. *Colloids and surfaces B: Biointerfaces, 75*(1), 335—341.

Vogel, M., Fischer, S., Maffert, A., Hübner, R., Scheinost, A. C., Franzen, C., & Steudtner, R. (2018). Biotransformation and detoxification of selenite by microbial biogenesis of selenium-sulfur nanoparticles. *Journal of hazardous materials, 344*, 749—757.

Wang, H., Zhang, J., & Yu, H. (2007). Elemental selenium at nano size possesses lower toxicity without compromising the fundamental effect on selenoenzymes: comparison with selenomethionine in mice. *Free Radical Biology and Medicine, 42*(10), 1524—1533.

Zhang, J., Wang, X., & Xu, T. (2008). Elemental selenium at nano size (Nano-Se) as a potential chemo preventive agent with reduced risk of selenium toxicity: comparison with Se-methylselenocysteine in mice. *Toxicological sciences, 101*(1), 22—31.

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ДОСЛІДЖЕННЯ ПРОБІОТИЧНИХ БАКТЕРІЙ РОДУ *BACILLUS (B. CLAUSII)* ЩОДО БІОГЕННОГО ЕКСТРАЦЕЛЮЛЯРНОГО СИНТЕЗУ НАНОЧАСТИНОК СЕЛЕНУ

Біогенний метод синтезу наночастинок за участі мікроорганізмів, які мають здатність виробляти наноматеріали різної форми, розміру та хімічного складу, є перспективним інноваційним напрямком нанотехнологій. Бактерії обираються для виробництва наночастинок завдяки їх швидкому розмноженню, простоті вирощування, низьким енергетичним потребам і мінімальним витратам. Складні синтетичні механізми, доступні мікроорганізмам, дозволяють їм використовувати велику кількість будівельних блоків для побудови нових біосинтетичних наноструктур, які можуть накопичуватися у везикулах всередині клітини, або шляхом екстрацелюлярного синтезу. У сучасному світі на перше місце виходять так звані «зелені» технології, проводиться активне вивчення та використання мікроорганізмів, що мають високий ферментативний потенціал, можуть використовуватися в нанобіотехнології та є перспективними для практичного застосування. Нами було проведено скринінг штамів бактерій роду *Bacillus* за здатністю до редукції Se (IV) у складі селеніту натрію до Se^0. **Мета.** Дослідити процеси біогенного синтезу наночастинок селену пробіотичними штамами *Bacillus clausii* та їх перспективи щодо практичного застосування. **Методи.** Культивування *B. clausii* проводили у флаконах (500 $см^3$) на ротаційному шейкері (20 об/хв.) за температури 30 °С впродовж 3-х діб на поживному середовищі МПБ. У середовище додатково вносили селеніт натрію 0.0065 г/100 мл. Проводили візуальну оцінку зміни кольору поживного середовища культури за умов його збагачення 30 ppm Se у складі селеніту натрію. Характеристики Nano-Se вивчали за допомогою трансмісійної електронної мікроскопії (ТЕМ). **Результати.** Внесення селеніту натрію 0.0065 г/100 мл (30 ppm Se) до поживного середовища виявило здатність *B. clausii* до редукції оксианіонів Se (IV) у наночастинки елементарного селену (Se^0) та утворення помаранчевого забарвлення. Проводили сепарацію бактеріальних клітин та біосинтезованих наночастинок селену для подальшого ТЕМ аналізу. На ТЕМ-зображеннях виявлено синтезовані нанокристали Nano-Se. Розміри частинок Nano-Se, визначені з ТЕМ зображень, варіюють у межах 298 ± 52 нм. Наночастинки, утворені *B. clausii*, формують конгломерати нанокристалів; окремі наночастинки мають сферичну форму. Зміну кольору середовища під впливом Na_2SeO_3 при культивуванні *B. clausii* відмічали при переході від логарифмічної фази зростання культур до стаціонарної. Дослідженнями вперше встановлено, що *B. clausii* здатні до редукції селеніту до елементарного селену, про що свідчать дані ТЕМ. **Висновки.** Отримані дані свідчать про здатність *B. clausii* до редукції селеніту натрію з утворенням позаклітинних наночастинок селену (Nano-Se). Трансформація селеніту натрію під впливом *B. clausii* відкриває доступне джерело біогенного Nano-Se для створення селеновмісних пробіотичних препаратів на його основі.

Ключові слова: *біогенний синтез, селеніт натрію, наночастинки селену, пробіотичні штами, Bacillus clausii, ТЕМ, позаклітинний синтез.*