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THE EFFECT OF NANOCERIA ON THE PROBIOTIC PROPERTIES OF *BACILLUS AMYLOLIQUEFACIENS* ssp. *PLANTARUM* STRAINS

A novel approach to the therapeutic enhancement of probiotics is their integration with distinctive (having a positive effect on living organisms) nanoparticles. The ***aim*** *of the study was to examine the impact of nanoceria (nano-CeO_2) on the growth of probiotic bacilli, as well as on their antimicrobial activity and antioxidant potential, with the intention of developing a biocomposite preparation for the prevention and treatment of infections.* ***Methods.*** *The number of viable bacterial cells was determined by the tenfold dilution method. The antimicrobial activity of probiotics was evaluated by disk diffusion, well diffusion tests, and measurement of growth inhibition zones of test cultures (Proteus vulgaris, Escherichia coli, Staphylococcus aureus, Candida albicans, Pseudomonas aeruginosa). The antioxidant potential of cell-free supernatants of probiotic bacteria was studied using spectrophotometric methods.* ***Results.*** *The growth activity of probiotic strains Bacillus amyloliquefaciens ssp. plantarum IMV B-7142 and Bacillus amyloliquefaciens ssp. plantarum IMV B-7143 reached its maximum when grown with 0.01 mM nano-CeO_2. This concentration was determined to be optimal. When testing the antimicrobial activity of probiotic bacilli under the influence of nano-CeO_2, its stimulating effect was revealed, but to a different degree for each of the two studied strains. B. amyloliquefaciens ssp. plantarum IMV B-7142 inhibited the growth of opportunistic pathogenic test cultures more actively than strain IMV B-7143. The antioxidant potential of cell-free supernatants of the studied bacteria was enhanced by nano-CeO_2, although to varying degrees. In particular, the activity of hydroxyl radical scavenging markedly increased compared to the control.* ***Conclusions.*** *Consequently, nano-CeO_2, due to its stimulatory effect on the physiological and biochemical activity of B. amyloliquefaciens ssp. plantarum IMV B-7142 and IMV B-7143, may be a promising component of a biocomposite preparation.*

Keywords: *Bacillus amyloliquefaciens ssp. plantarum, cerium dioxide nanoparticles, antimicrobial activity, antioxidant potential.*

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The development of nanotechnology has led to the emergence of a research area focused on the creation of biocomposite preparations based on probiotic bacterial strains and various nanomaterials. These preparations exhibit anti-cancer, antimicrobial, antioxidant, and photoprotective properties (Dangi et al., 2023). Among probiotics, an important group is represented by bacteria of the genus *Bacillus*, which have gained significant interest over the past 20 years. This is evidenced by the accumulation of scientific data demonstrating their preventive and therapeutic efficacy in diseases of the gastrointestinal tract and metabolic and immune status disorders (Cutting, 2011; Jezewska-Frackowiak et al., 2018; Kieps et al., 2022; Lubkowska et al., 2023; Williams et al., 2024).

The high antagonistic activity of *Bacillus* bacteria against a wide range of pathogenic and opportunistic microorganisms is directly related to their ability to produce a wide range of antimicrobial substances (Sumi et al., 2015). This is one of the most promising groups of microorganisms for the development of therapeutic and prophylactic preparations for humans and animals.

A number of studies have demonstrated significant interest in probiotics as a means of treating infectious diseases and an alternative to antibiotics. This is because the irrational use of antibiotics in recent decades has led to the emergence and spread of antibiotic-resistant bacteria worldwide, which poses a significant threat to human health and well-being (Silva et al., 2020). Therefore, there is an urgent need to develop new strategies to combat antibiotic resistance.

The potential of probiotics as an alternative to antibiotics is increasingly considered, given the irrational use of antibiotics in recent decades, which has led to the emergence and spread of antibiotic-resistant bacteria worldwide. This poses a real threat to human health and well-being (Silva et al., 2020). Therefore, there is an urgent need to develop new strategies to combat antibiotic resistance.

The probiotic strains of bacteria *Bacillus amyloliquefaciens* ssp. *plantarum* IMV B-7142 and *B. amyloliquefaciens* ssp. *plantarum* IMV B-7143 serve as the basis for a veterinary biological product designed for the treatment and prevention of dysbiosis, intestinal and purulent infections, postpartum endometritis, and fetal membranes (afterbirth) retention (Safronova et al., 2006; Safronova et al., 2009; Safronova et al., 2012). These strains are distinguished by their pronounced antimicrobial properties against a diverse range of microorganisms isolated from various environments, as well as by their immunomodulatory and antioxidant properties (Didenko et al., 2013; Safronova, 2015; Safronova et al., 2021). Consequently, we have selected these bacillus strains as promising microorganisms for the development of a biocomposite preparation for the prevention and treatment of infections.

Among the nanomaterials with significant potential in biomedical research, cerium dioxide nanoparticles (CeO_2NPs) deserve particular attention. The biologically significant properties of CeO_2NPs are due to the thermodynamic balance of the redox potential of Ce^{3+}/Ce^{4+} on their surface (Sadidi et al., 2020) and the ability to absorb oxygen or form oxygen vacancies (Deshpande et al., 2005). Moreover, the biological effects of nano-CeO_2 are closely related to the method of nanoparticles preparation, their size, degree of dispersion, and the characteristics of the dispersed medium itself (Dhall et al., 2018).

Nano-CeO_2 displays significant antimicrobial activity against both gram-positive and gram-negative bacterial strains. The antimicrobial activity of nano-CeO_2 has been demonstrated against a range of pathogenic and opportunistic bacteria, including *Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Salmonella typhimurium, Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumonia, Enterobacter* spp., and *Acinetobacter baumannii* (Farias et al., 2018; Zhang et al., 2019; Barker et al., 2022). CeO_2NPs are characterized by antiviral activity, demonstrating the ability to protect cells from RNA- and DNA-containing viruses both

before and after infection (Zholobak et al., 2011; Shcherbakov et al., 2011; Shcherbakov et al., 2020; Shcherbakov et al., 2024). Nano-CeO_2 demonstrates a high antioxidant potential. In particular, it displays the antioxidant enzyme-mimetic activities (catalase (Rzigalinski et al., 2006), peroxidase (Jiao et al., 2012), superoxide dismutase (Korsvik et al., 2007), scavenging free radicals and, as a consequence, alleviating oxidative stress (Kargozar et al., 2018). In addition to the aforementioned properties, CeO_2NPs possess unique biofunctions that include anti-inflammatory, angiogenic, and anti-apoptotic ones (Sadidi et al., 2020).

The integration of probiotics with nanomaterials has the potential to be a highly promising area of nanobiotechnology (Dangi et al., 2023; Pandey et al., 2024; Sadeghi et al., 2023). However, one significant drawback of this field is the limited number of studies on the effect of nanoparticles on the physiological and biochemical activity of probiotic bacteria. A comprehensive understanding of the interaction between NPs and probiotics at the nano-biological interface is a crucial component in the development of biocomposite preparations and a deeper understanding of their effectiveness.

Accordingly, the **aim** of this work was to examine the impact of nano-CeO_2 on the probiotic properties (antimicrobial activity and antioxidant potential) of *B. amyloliquefaciens* ssp. *plantarum* IMV B-7142 and IMVB-7143 strains with further evaluation of the prospects for the development of a preparation for the prevention and treatment of infections based on them.

Materials and Methods. ***Nano-CeO_2 characterization.*** In this study, we employed a citrate-stabilized nano-CeO_2 sol (0.1 M, 3—4 nm particle size, 1:1 stabilizer-to-nano-CeO_2 ratio). This sol was synthesized by A.V. Shcherbakov, PhD in chemistry, senior researcher (Zabolotn**y** Institute of Microbiology and Virology, National Academy of Sciences of Ukraine). The citrate-stabilized aqueous solution of CeO_2 was prepared as described in (Ivanov et al., 2010). For the experimental studies, the concentration of nano-CeO_2 effluent sol was altered by the addition of appropriate volumes to sterile saline, after which the resulting aliquots were added to the medium for the growth of microorganisms in a ratio of 1:100 (v/v).

Probiotic strains and conditions of their cultivation. Bacterial strains *B. amyloliquefaciens* ssp. *plantarum* IMV B-7142 and IMV B-7143 were cultivated in synthetic medium of the following composition (g/L): glucose — 15.2; sodium citrate — 1.29; $(NH_4)_2HPO_4$ — 4.75; KH_2PO_4 — 9.63; $MgSO_4 \times 7H_2O$ — 0.18; distilled water — 1 L; pH = 6.4—7.0. The bacilli were cultured under batch conditions with shaking at 200 rpm in Erlenmeyer flasks of 750 mL volume containing 50 mL of the nutrient medium. The inoculum was 5%, which was prepared from 24 h of bacterial cells after growth on meat-peptone agar (MPA) according to the density standard of 5 units. The growing time was 24—72 h at a temperature of 37 ± 1 °C. The number of viable cells (colony-forming units, CFU/mL) was determined by serial tenfold dilutions in saline followed by inoculation on MPA. The pH of the nutrient medium was determined potentiometrically. Nano-CeO_2 was added to the culture medium at a final concentration of 1 mM, 0.1 mM, and 0.01 mM.

Opportunistic pathogenic strains of microorganisms. The following test cultures were used in this study: *P. vulgaris* 72, *E. coli* 028, *S. aureus* 209, *Candida albicans* 690, and *P. aeruginosa* ATCC 27959, which were obtained from the Collection of Microorganisms of the Department of Antibiotics of the Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine.

Preparation of the cell-free supernatants (CFS) of probiotic strains. The cell-free supernatants (CFS) of the probiotic strains *B. amyloliquefaciens* ssp. *plantarum* were obtained by centrifugation of bacteria culture liquids on an OPn-8 centrifuge (UJSC TNK DASTAN, Kyrgyzstan) at 5000 g for 15 min. The cell-free supernatants were passed through syringe filters (PTFE, hydrophilic, d = 13 mm, membrane pore

size = 0.22 μm) and used to assess their antimicrobial activity and antioxidant potential.

Screening of CFS for antimicrobial activity. The antimicrobial activity was determined by the agar well diffusion method (Balouiri et al., 2016). To this end, opportunistic pathogenic test cultures were grown on MPA for 20—24 hours. The cell suspensions of these microorganisms were prepared in saline according to the density standard of 5 units, and 0.2 mL was platted on the agar medium, the composition of which was as follows (g/L): Hottinger broth — 30 mL; enzymatic peptone — 5 g; NaCl — 5 g; glucose — 10 g; agar-agar — 20 g; distilled water — 1 L; pH = 6.7—7.0. Under aseptic conditions, 0.8 cm in diameter wells were made, where 50 μL of cell-free supernatant of the culture liquid of each bacillus strain was poured. All experiments were performed in triplicate. Incubation was conducted at 37 ± 1 °C for 24—72 hours. The diameter of the zone of inhibition of the growth of opportunistic pathogens by the supernatant of each of the studied bacillus strains was determined.

Evaluation of the antioxidant potential of cell-free supernatants of the strains B. amyloliquefaciens ssp. plantarum. DPPH·-free radical scavenging activity. The antiradical activity (ARA) of the CFS of probiotic strains was determined by the method (Shimada et al., 1992), using the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH·). The interaction of antioxidants (AN) with DPPH· is based on the donor-acceptor mechanism. The radical accepts a labile hydrogen atom from the antioxidant to form a stable diamagnetic molecule: DPPH· + AN → DPPH-H + A· in compliance with the law of charge conservation.

Briefly, 1 mL of 0.1 mM ethanolic DPPH• solution was added to the glass tubes, mixed with 3 mL of the CFS of the test strain of bacilli, and left at room temperature for 30 minutes. A control sample instead of the CFS of bacilli contained 3 mL of the nutrient medium. The intensity of the change of the violet color of the methanolic solution DPPH• to a bright yellow color was recorded at a wavelength of 517 nm using a UV-46 spectrophotometer. The decrease in the absorbance of the test samples indicated their high ARA relative to DPPH•. The percent of the DPPH• scavenging effect was calculated using the following expression:

$$\text{DPPH• scavenging effect (\%)} = [1 - (A_{sample}/A_{control})] \cdot 100\ \%,$$

where A_{sample} is the absorbance in the presence of the CFS of *B. amyloliquefaciens* ssp. *plantarum* IMV B-7142 or *B. amyloliquefaciens* ssp. *plantarum* IMV B-7143 and $A_{control}$ is the absorbance of the control. The control contained all reagents except the CFE of the investigation strains of bacilli. All tests were performed in triplicate, and means were determined.

Reducing Power. The reducing power of the CFS of *B. amyloliquefaciens* ssp. *plantarum* strains was determined by the Oyaizu method (Oyaizu, 1986). Their ability to reduce $K_3[Fe^{3+}(CN)_6]$ to $K_4[Fe^{2+}(CN)_6]$ (potassium hexacyanoferrate (II)) at a wavelength of 700 nm was determined. An increase in the absorption of the reaction mixture indicated an increase in the reducing ability.

Chelating Activity on Fe^{2+}. The metal-chelating activity of the CFS of probiotic strains was determined by the method described in (Dinis et al., 1994) with certain modifications. The change in the color intensity of the ferrozine complex with iron ions allows us to estimate the ability to bind metal ions of variable valence by compounds contained in the biological material under study.

Superoxide radical scavenging activity ($O_2^{\bullet-}$). This activity was measured by the reduction of nitroblue tetrazolium by a previously described method (Fontana et al., 2001). The non-enzymatic phenazine-methosulfate-nicotinamide-adenine dinucleotide system generates superoxide radicals which reduce nitroblue tetrazolium to purple formazan.

Nitric oxide (NO) scavenging activity. At physiological pH, nitric oxide formed from an aqueous solution of sodium nitroprusside reacts with oxygen to form nitrite ions, which can

be quantified using the Griess Illosvoy reaction (Bruan et al., 2007).

Hydroxyl radical scavenging activity (·OH). The ability of the CFS of probiotic strains to scavenge of ·OH was determined by the method of (Halliwell et al., 1981). The absorbance of the reaction mixture was measured at a wavelength of 532 nm.

DMPD·$^+$ scavenging activity. The methodology employed in this study was derived from the approach described in(Fogliano et al., 1999). The principle of the DMPD$^{\cdot+}$ assay is that at an acidic pH and in the presence of an appropriate oxidant solution, DMPD (N, N-dimethyl-p-phenylendiamine) can form a stable and colored radical cation (DMPD$^{\cdot+}$). Antioxidant compounds which are able to transfer a hydrogen atom to DMPD$^{\cdot+}$ cause the solution to discolor. This reaction is rapid, and the stable endpoint is taken as a measure of antioxidant efficiency.

Statistical analysis. Methods of variation statistics were used for statistical analysis of research results, in particular determination of the arithmetic means (X), their standard deviations (SD), and standard errors (m) (Barde et al., 2012). Results were presented as the means ± standard deviation of triplicate experiments. Statistical data processing was also performed using the computer program STATISTICA 6.0.

Results. ***Effect of different concentrations of nano-CeO2 on the growth of probiotic strains of B. amyloliquefaciens ssp. plantarum.*** At the initial stage of the study, the main task was to determine the effect of different concentrations of nano-CeO_2 on the growth of *B. amyloliquefaciens* ssp. *plantarum* IMV B-7142 and IMV B-7143 strains. It was found that the optimum concentration of nano-CeO_2 in the nutrient medium was 0.01 mM, at which the growth of *B. amyloliquefaciens* ssp. *plantarum* IMV B-7142 increased by 33%, and *B. amyloliquefaciens* ssp. *plantarum* IMV B-7143 — by 18%, compared to the control. This concentration of nano-CeO_2 was used in the following studies of the antimicrobial activity and antioxidant potential of cell-free supernatants of these probiotic bacillus strains (Table 1).

When strain *B. amyloliquefaciens* ssp. *plantarum* IMV B-7142 was grown for 24—48 h without nano-CeO_2, the pH of the culture medium decreased from 6.4 to 6.1. However, when this strain was cultured under similar conditions but with 0.01 mM nano-CeO_2, the pH of the medium decreased to 5.9 (Table 2). At the same time, when *B. amyloliquefaciens* ssp. *plantarum* IMV B-7143 was cultured with and without nano-CeO_2 for 24 h, the pH of the medium decreased from 6.4 to 5.7—5.6, and after 48 h of cultiva-

Table 1. **Growth of the bacterial strains *B. amyloliquefaciens* ssp. *plantarum* IMV B-7142 and *B. amyloliquefaciens* ssp. *plantarum* IMV B-7143 during cultivation with different concentrations of nano-CeO_2**

Sample	Number of viable cells (CFU/mL)	
	B. amyloliquefaciens ssp. *plantarum* IMV B-7142	*B. amyloliquefaciens* ssp. *plantarum* IMV B-7143
*Control	$(2.65 \pm 0.1) \cdot \times 10^8$	$(3.29 \pm 0.3) \times 10^8$
+ 1 mM nano-CeO_2	$(9.67 \pm 0.2) \cdot \times 10^6$	$(1.37 \pm 0.2) \times 10^7$
+ 0.1 mM nano-CeO_2	$(2.03 \pm 0.2) \cdot \times 10^8$	$(1.76 \pm 0.1) \times 10^8$
+ 0.01 mM nano-CeO_2	$(3.52 \pm 0.1) \cdot \times 10^8$	$(3.88 \pm 0.3) \times 10^8$

The initial concentration of *B. amyloliquefaciens* ssp. *plantarum* IMV B-7142 cells was $(5.10 \pm 0.5) \times 10^6$ CFU/mL; The initial concentration of *B. amyloliquefaciens* ssp. *plantarum* IMV B-7143 cells was $(1.29 \pm 0.1) \times 10^6$ CFU/mL; *Control — nutrient medium without nano-CeO_2.

tion, the value of pH was 5.2 (Table 2). It can be assumed that such differences in pH values are due to the different metabolic profiles of the bacterial cultures studied, which differ in the biological activity. According to Table 2, the most pronounced decrease in pH values was observed during the growth of the IMV B-7143 strain.

After the first day of cultivation of bacteria in the nutrient medium with nano-CeO_2, the growth of the IMV B-7142 strain increased by 63.6% and that of the IMV B-7143 strain by 100%. On the second day of cultivation, the presence of such a concentration of nano-CeO_2 in the medium did not lead to any significant changes in the growth activity of the bacilli. Therefore, in further experiments, the cultures were grown in the medium containing 0.01 mM nano-CeO_2 for 24—48 h.

Determination of the antimicrobial activity of cell-free supernatants of probiotic bacillus strains when cultivated with nano-CeO_2. According to the results of studies of the inhibitory effect of cell-free supernatants of the IMV B-7142 and IMV B-7143 strains on the growth of opportunistic pathogenic test cultures, strain differences were found. Such effects of both studied strains against a wide range of opportunistic bacteria are due to their ability to synthesize various compounds with pronounced antimicrobial properties.

It was observed that the antimicrobial activity of the CFS of *B. amyloliquefaciens* ssp. *plantarum* IMV B-7142, against all test cultures increased after 72 h of cultivation, but remained practically unchanged against the yeast-like fungus *C. albicans* 690 (Table 3). Upon examination of the antimicrobial activity of the CFS of the 3-day culture of the IMV B-7142 strain, it was found that the level of inhibition of *P. vulgaris* 72 increased by 21.7%, *E. coli* 028 — by 22.9%, and *S. aureus* 209 — by 9.6%, compared to the antimicrobial activity of the CFS of the one-day culture. Herein, zones of inhibition of *P. aeruginosa* ATCC 27959 appeared (Table 3).

A different pattern was observed for strain *B. amyloliquefaciens* ssp. *plantarum* IMV B-7143. Thus, after 72 h of cultivation of this strain, the antimicrobial activity of its CFS increased by 4% against *P. vulgaris* 72 and by 45.5%against *S. aureus* 209, and zones of inhibition of *P. aeruginosa* ATCC 27959 appeared, compared with the antimicrobial activity of the CFS of one-day culture. At the same time, the activity against *E. coli* 028 decreased by 34.2% and against *C. albicans* 690 — by 23.1% (Table 4). According to the results obtained, the higher level of antimicrobial activity was demonstrated by *B. amyloliquefaciens* ssp. *plantarum* IMV B-7142.

It was found that the nutrient medium with nano-CeO_2 at a final concentration of 0.01 mM did not show antimicrobial action against the studied strains of opportunistic pathogens, whereas a different trend was observed during the co-cultivation of probiotic bacilli with CeO_2NPs**.**

In particular, the CFS of the IMV B-7142 strain that was obtained after 24 h of bacterial

Table 2. **Growth characteristics of *B. amyloliquefaciens* ssp. *plantarum* IMV B-7142 and IMV B-7143 when grown with 0.01 mM nano-CeO_2 for different time periods**

Cultivation period, h	*B. amyloliquefaciens.* ssp. *plantarum* IMV B-7142				*B.* amyloliquefaciens. ssp. *plantarum* IMV B-7143			
	Control* (CFU/mL)	pH	+ 0.01 mM nano-CeO_2 (CFU/mL)	pH	Control* (CFU/mL)	pH	+ 0.01 mM nano-CeO_2 (CFU/mL)	pH
0	$(3.9 \pm 0.3) \times 10^6$	6.4	$(3.9 \pm 0.3) \times 10^6$	6.4	$(3.9 \pm 0.3) \times 10^6$	6.4	$(3.9 \pm 0.3) \times 10^6$	6.4
24	$(1.1 \pm 0.1) \times 10^9$	6.1	$(1.8 \pm 0.1) \times 10^9$	6.0	$(1.5 \pm 0.2) \times 10^9$	5.6	$(3.0 \pm 0.1) \times 10^9$	5.7
48	$(1.3 \pm 0.1) \times 10^9$	6.1	$(1.4 \pm 0.1) \times 10^9$	5.9	$(2.0 \pm 0.2) \times 10^9$	5.2	$(1.9 \pm 0.1) \times 10^9$	5.2

*Control —nutrient medium without nano-CeO_2.

cultivation with CeO_2NPs demonstrated significant inhibitory activity against the growth of cells of opportunistic pathogenic test cultures, except for *P. aeruginosa* ATCC 27959. This activity was higher than that found in this strain without the addition of nano-CeO_2 (Table 5).

The CFS of the IMV B-7142 strain obtained after 48 h of bacterial cultivation with nano-CeO_2 demonstrated an even higher level of antimicrobial activity against all the test cultures studied. The zones of growth inhibition of *P. vulgaris* 72 strains increased by 51.7%, *E. coli* 028 — by 35.3%, *S. aureus* 209 — by 58.2%, and *C. albicans* 690 — by 59.1%. The exception was the antimicrobial activity against *P. aeruginosa* ATCC 27959. The diameter of the zones of growth inhibition of this strain was at the level of the variant without the addition of nano-CeO_2 (Table 5).

The cultivation of the IMV B-7143 strain with nano-CeO_2 for 24 hours resulted in increasing the antimicrobial activity of its CFS against opportunistic pathogenic test cultures. However, this was not observed for *P. aeruginosa* ATCC 27959. Furthermore, the absence of an inhibitory effect against this strain under the conditions of this experiment was also observed in the absence of nano-CeO_2 during cultivation. The addition of nano-CeO_2 to the nutrient medium had a less pronounced effect on the antimicrobial activity of the probiotic strain IMV B-7143 compared to the strain IMV B-7142. In this case, the diameter of the zones of growth inhibition of *P. vulgaris* 72 increased by 32.5%, *E. coli* 028 — by 15.6%, *S. aureus* 209 — by 5.9%, and *C. albicans* 690 — by 12.0% compared to the control (Table 5). After 48 h of cultivation of this strain in the nutrient medium with nano-CeO_2, the an-

Table 3. **Antimicrobial activity of cell-free supernatants of *B. amyloliquefaciens* ssp. *plantarum* IMV B-7142 obtained after 24—72 hours of bacterial cultivation**

Conditionally pathogenic strains of microorganisms	Diameter of the test culture inhibition zones, mm		
	24 h	48 h	72 h
P. vulgaris 72	11.5 ± 0.7	15.0 ± 2.3	14.0 ± 1.2
E. coli 028	14.0 ± 1.4	18.5 ± 0.7	17.2 ± 1.1
S. aureus 209	12.5 ± 0.7	14.8 ± 0.7	13.7 ± 0.7
C. albicans 690	11.5 ± 0.7	12.4 ± 0.7	11.3 ± 0.81
P. aeruginosa ATCC 27959	—	12.5 ± 0.7	11.8 ± 0.8

«—» —There is no inhibition zone.

Table 4. **Antimicrobial activity of the cell-free supernatant of *B. amyloliquefaciens* ssp. *plantarum* IMV B-7143 obtained after 24—72 hours of bacterial cultivation**

Conditionally pathogenic strains of microorganisms	Diameter of the test culture inhibition zones, mm		
	24 h	48 h	72 h
P. vulgaris 72	12.5 ± 0.7	14.5 ± 2.1	13.0 ± 1.5
E. coli 028	15.5 ± 0.7	11.5 ± 0.7	10.2 ± 0.5
S. aureus 209	11.0 ± 0.0	17.5 ± 0.7	16.0 ± 0.6
C. albicans 690	13.0 ± 0.0	9.5 ± 2.1	10.0 ± 0.5
P. aeruginosa ATCC 27959	—	13.0 ± 1.4	13.5 ± 0.7

«—» — There is no inhibition zone.

timicrobial activity of its CFS increased by 29.8% against *P. vulgaris* 72, *E. coli* 028 — by 20.0%, *S. aureus* 209 — by 17.5%, *C. albicans* 690 — by 67.2%, and *P. aeruginosa* ATCC 27959 — by 21.2%, compared to the control (Table 5).

Evaluation of the antioxidant potential of cell-free supernatants of probiotic strains of B. amyloliquefaciens ssp. plantarum during their cultivation with nano-CeO_2. We have found that the cultivation of probiotic strains *B. amyloliquefaciens* spp. *plantarum* IMV B-7142 and IMV B-7143 with 0.01 mM nano-CeO_2 for 24—48 h significantly increased the ability of their cell-free supernatants to inactivate DPPH·. Accordingly, the ARA values for the CFS of the IMV B-7142 strain increased to 71.3 — 86.3% (Table 6) and for the CFS of IMV B-7143 strain — to 61.2 — 66.8% (Table 7).

We observed pronounced changes in the reducing capacity of the cell-free supernatants of the studied probiotic bacteria grown with nano-CeO_2. It was shown that the studied index of antioxidant potential for the CFS of the IMV B-7142 strain reached high values during its cultivation with nano-CeO_2 for 24—48 h (Table 6). On the other hand, the reducing capacity of the CFS of the IMV B-7143 strain decreased after 24 h of cultivation with nano-CeO_2 compared to the control. However, after 48 h of cultivation in the presence of nano-CeO_2, it reached high values again (Table 7). This may indicate that the active synthesis of compounds with a high redox potential by the probiotic strains occurs after 48 h of cultivation.

The addition of nano-CeO_2 into the culture medium significantly affected the metal chelating activity of the cell-free supernatants of the studied probiotics. For example, this indicator reached the highest values (81.8% and 83.1%) for cell-free supernatants of IMV B-7142 and IMV B-7143 strains after 24 h of bacterial cultivation with nano-CeO_2. As the cultivation time of the probiotic cultures increased, the metal

Table 5. **Antimicrobial activity of cell-free supernatants of *B. amyloliquefaciens* ssp. *plantarum* strains IMV B-7142 and IMV B-7143 that was obtained after cultivation in nutrient medium with nano-CeO_2 (0.01mM) for 24—48 hours**

Conditionally pathogenic strains of microorganisms	Diameter of the test culture inhibition zones, mm			
	B. amyloliquefaciens ssp. *plantarum* IMV B-7142		*B. amyloliquefaciens* ssp. *plantarum* IMV B-7143	
	Control 24 h	+ nano-CeO_2 24 h	Control 24 h	+ nano-CeO_2 24 h
P. vulgaris 72	12.7 ± 0.9	14.0 ± 1.3	11.7 ± 0.6	15.5 ± 0.9**
E. coli 028	15.1 ± 1.2	17.5 ± 0.6*	14.1 ± 0.9	16.3 ± 0.5*
S. aureus 209	11.8 ± 0.7	19.5 ± 0.3**	11.8 ± 0.3	12.5 ± 0.6
C. albicans 690	11.7 ± 0.5	16.5 ± 1.1**	12.5 ± 0.5	14.0 ± 1.4
P. aeruginosa ATCC 27959	—	—	—	—
	48 h	48 h	48 h	48 h
P. vulgaris 72	14.5 ± 2.1	22.0 ± 2.6**	13.1 ± 2.4	17.0 ± 1.8
E. coli 028	17.0 ± 0.5	23.0 ± 0.1**	12.5 ± 0.6	15.0 ± 1.1*
S. aureus 209	15.8 ± 0.8	25.0 ± 4.4**	18.3 ± 0.9	21.5 ± 0.9*
C. albicans 690	13.2 ± 0.6	21.0 ± 4.1**	11.6 ± 0.7	19.4 ± 0.7**
P. aeruginosa ATCC 27959	11.3 ± 0.3	13.2 ± 0.8*	11.8 ± 0.6	14.3 ± 0.4*

«—» —There is no inhibition zone; * $P<0.05$; ** $P<0.01$; Control — a cell-free supernatant that was obtained after cultivation of a specific strain of probiotic bacteria in a nutrient medium without the addition of nano-CeO_2.

chelating activity for their cell-free supernatants decreased (Tables 6 and 7).

It was found that during the cultivation of probiotic strain IMV B-7142 with nano-CeO_2, the superoxide anion radical ($O_2^{\bullet-}$) scavenging activity of its CFS increased only after 24 h and amounted to 54.2%, while after 48 h it decreased to 37.8% (Table 6). The CFS of the IMV B-7143 strain was characterized by high rates of this antiradical activity regardless of the cultivation time (Table 7).

Nano-CeO_2 did not significantly affect the nitric oxide radical (NO·) scavenging activity for cell-free supernatants of both studied probiotic

Table 6. **Antioxidant potential of the cell-free supernatant of probiotic strain *B. amyloliquefaciens* ssp. *plantarum* IMV B-7142 for cultivation of bacteria with nano-CeO_2 (0.01 mM) for 24—48 hours**

Indexes of antioxidant potential	Samples			
	Control* 24 h	+ nano-CeO_2** 24 h	Control* 48 h	+ nano-CeO_2** 48 h
DPPH··-free radical scavenging activity,%	46.2 ± 5.5	71.3 ± 1.9	83.6 ± 2.2	86.3 ± 2.4
Reducing power	0.241 ± 0.003	0.692 ± 0.004	0.370 ± 0.011	0.758 ± 0.025
Chelating activity on Fe^{2+},%	79.7 ± 3.2	81.8 ± 5.8	22.2 ± 4.6	48.3 ± 1.3
Superoxide radical scavenging activity ($O_2^{\bullet-}$),%	53.4 ± 1.9	54.2 ± 2.6	52.1 ± 5.4	37.8± 3.5
Nitric oxide (NO) scavenging activity,%	53.6 ± 4.0	52.9 ± 1.1	22.6 ± 2.5	31.8 ± 8.6
DMPD·$^+$ scavenging activity,%	64.5 ±2.8	64.6 ± 1.4	60.3 ± 3.8	59.2 ± 1.7
Hydroxyl radical scavenging activity (·OH),%	77.8 ± 5.6	88.9 ± 8.9	83.8 ± 6.4	95.8 ± 4.7

*Control — the cell-free supernatant of the probiotic strain *B. amyloliquefaciens* ssp. *plantarum* IMV B-7142 obtained after cultivation of bacteria without nano-CeO_2

** + nano-CeO_2 — the cell-free supernatant of the probiotic strain *B. amyloliquefaciens* ssp. *plantarum* IMV B-7142 obtained after cultivation of bacteria with nano-CeO_2.

Table 7. **Antioxidant potential of the cell-free supernatant of probiotic strain *B. amyloliquefaciens* ssp. *plantarum* IMV B-7143 for cultivation of bacteria with nano-CeO_2 (0.01 mM) for 24—48 hours**

Indexes of antioxidant potential	Samples			
	Control* 24 h	+ nano-CeO_2** 24 h	Control* 48 h	+ nano-CeO_2** 48 h
DPPH··-free radical scavenging activity,%	55.1 ± 3.5	61.2 ± 1.2	64.9 ± 2.4	66.8 ± 4.8
Reducing power	1.004 ± 0.003	0.495 ± 0.004'	0.487 ± 0.008	0.867 ± 0.012'
Chelating activity on Fe^{2+},%	65.9 ± 3.6	83.1 ± 5.6'	3.5 ± 0.6	5.2 ± 0.3'
Superoxide radical scavenging activity ($O_2^{\bullet-}$),%	70.7 ± 1.4	77.1 ± 3.9'	83.8 ± 7.2	74.8 ± 4.5
Nitric oxide (NO) scavenging activity,%	72.4 ± 4.8	64.1 ± 1.9'	67.0 ± 2.9	66.9 ± 6.6
DMPD·$^+$ scavenging activity,%	62.7 ± 2.4	59.3 ± 1.8	59.7 ± 6.8	56.5 ± 1.4
Hydroxyl radical scavenging activity (·OH),%	82.5 ± 4.4	89.9 ± 7.3	78.8 ± 3.1	93.3 ± 5.7'

*Control — the cell-free supernatant of probiotic strain *B. amyloliquefaciens* ssp. *plantarum* IMV B-7143 obtained after cultivation of bacteria without nano-CeO_2

** + nano-CeO_2 — the cell-free supernatant of probiotic strain *B. amyloliquefaciens* ssp. *plantarum* IMV B-7143 obtained after cultivation of bacteria with nano-CeO_2.

strains. The same effect of nano-CeO_2 was observed in the study of N.N-dimethyl-p-phenylenediamine cation radical ($DMPD^{\cdot+}$) scavenging activity (Tables 6, 7).

When probiotic strains *B. amyloliquefaciens* ssp. *plantarum* IMV B-7142 and IMV B-7143 were grown for 24—48 h in the presence of nano-CeO_2, their cell-free supernatants were characterized by a high ability to inactivate hydroxyl radical — by more than 80—90%, compared to control samples (Tables 6 and 7).

As seen, the cultivation of probiotic strains of *B. amyloliquefaciens* ssp. *plantarum* IMV B-7142 and IMV B-7143 in the presence of nano-CeO_2 (0.01 mM) enhanced the antioxidant potential of cell-free supernatants of these bacteria.

Discussion. The genus *Bacillus*, which is distinguished by its probiotic properties, is a globally recognized group of bacteria and has been shown to possess potential health benefits (Elshaghabee et al., 2017; Bahaddad et al., 2023). Due to their ability to form spores, bacilli are more stable during the processing and storage of foods and pharmaceuticals, rendering them a more suitable ingredient for health formulations (Grant et al., 2018).

A number of studies have demonstrated significant interest in probiotics as a treatment for infectious diseases. This is due to the emergence of a new problem in the world over recent decades: antibiotics developed to fight infectious diseases are becoming less effective because of the antibiotic resistance of pathogenic strains of microorganisms (Imperial et al., 2016).

The search for safe and cost-effective treatments, as well as the problem of increasing antibiotic resistance of opportunistic bacteria, has led researchers to look for an alternative to the current therapeutic regimens that are mainly antibiotic-dependent. Among the many options offered, probiotic therapy seems to be the most effective, with a long history of use and guaranteed safety.

The combination of probiotic strains with specific nanoparticles (NPs) can enhance their potential therapeutic and prophylactic effects, including antimicrobial action, especially if NPs have biologically significant activities (Alkushi et al., 2022). Among them, nano-CeO_2 is of great interest (Sadidi et al., 2020).

Nevertheless, current research on the combined use of probiotics and nanomaterials raises important questions. One of them concerns the effect of NPs on the physiological and biochemical properties of probiotic bacteria.

The study of the effect of different concentrations of CeO_2 NPs on the growth activity of probiotic strains *B. amyloliquefaciens* ssp. *plantarum* IMV B-7142 and IMV B-7143 revealed a concentration-dependent effect. The minimum millimolar concentration of (0.01 mM) had the greatest stimulating effect on the growth of the bacteria.

Chigurupati with co-authors (Chigurupati et al., 2012) obtained comparable results but on keratinocytes and fibroblasts. Both cell types exhibited enhanced growth rates following treatment with 1 mM and 10 mM of nano-CeO_2, compared to the control. Naganuma and colleagues (Naganuma et al., 2014) demonstrated that the valence states of Ce (Ce^{3+}/Ce^{4+}) play a significant role in inducing physiological cell functions.

Similar effects on the growth activity of bacteria have been obtained for other nanomaterials. According to the results of research (Meli et al., 2016), moderate concentrations of ZnO nanoparticles can accelerate the growth of some species of denitrifying bacteria. Palmgvist and co-authors (Palmgvist et al., 2015) observed an increase in the number of bacteria when *B. amyloliquefaciens* 5113 was co-cultured with nanotitanium. It has also been noted that nanogypsum (Chaudhary et al., 2019) and nanosilica (Karunakaran et al., 2013) can act as triggers for enhancing the growth of plant probiotics.

Our previous studies have shown that bacillus strains complement each other in terms of the spectrum and degree of antimicrobial activity against human, animal, and plant pathogens, as well as some other biological parameters. Previ-

ously, by the method of delayed antagonism on nutrient agar medium, it has been found that strain *B. amyloliquefaciens* ssp. *plantarum* IMV B-7142 has a high inhibitory effect against opportunistic bacteria (*S. aureus, E. coli, P. vulgaris, P. aeruginosa, Acinetobacter* spp.), phytopathogens (*P. syringae* pv. *atrofaciens*, *Agrobacterium tumefaciens, Clavibacter michiganensis* subsp. *michiganensis*), as well as various types of micromycetes, whereas the antimicrobial activity of strain *B. amyloliquefaciens* ssp. *plantarum* strain IMV B-7143 against these microorganisms was weaker (Safronova et al., 2012; Safronova et al., 2015).

Genome analysis of strain *B. amyloliquefaciens* ssp. *plantarum* IMV B-7143 using bioinformatics, HPLC, and MALDI-TOF-MS methods showed that the strain synthesizes antibiotics such as surfactin, fengycin, bacillaene, macrolactin D, and difficidin, as well as the siderophore bacillibactin (Reva et al., 2020). The ability to synthesize antibiotics is a special property of the *Bacillus* genus, and these compounds play a pivotal role in the mechanism of antimicrobial activity of bacteria (Tran et al., 2022).

Furthermore, it has been demonstrated that *B. amyloliquefaciens* ssp. *Plantarum* strains IMV B-7142 and IMV B-7143, when cultivated in a liquid nutrient medium, secrete a complex of bacteriolytic and yeastolytic enzymes into the nutrient medium. Among the test cultures studied, *E. coli* and *C. albicans* cells were lysed to a greater extent (Matseliukh et al., 2015). The capacity of *Bacillus* bacteria to destroy specific bonds within the peptidoglycan structure of the cell walls of various microorganisms also defines the antimicrobial properties of *Bacillus* strains (Tran et al., 2022).

The cultivation of probiotic strains of *B. amyloliquefaciens* ssp. *plantarum* IMV B-7142 and IMV B-7143 with 0.01 mM CeO_2NPs for 24—48 h resulted in increasing the antimicrobial activity of their cell-free supernatants. However, the strain specificity of probiotics under the influence of nano-CeO_2 was observed in terms of inhibition of the growth of conditionally pathogenic test cultures.

The antimicrobial potential of CeO_2NPs is influenced by both the method of synthesis and the spectrum of their physicochemical characteristics (Chatzimentor et al., 2023). For instance, CeO_2NPs, which were synthesized by the wet chemical synthesis method and had an average size of 5 to 15 nm, exhibited antimicrobial activity against five strains of pathogenic microorganisms: *Escherichia coli, Salmonella typhimurium, Listeria monocytogenes, Staphylococcus aureus,* and *Bacillus cereus* (Pop et al., 2020). Meanwhile, nano-CeO_2 synthesized by the hydrothermal method with an average particle size of 3-5 nm and a concentration of 50 μg/mL demonstrated antimicrobial effects against ESKAPE pathogens (*Enterobacter faecium, S. aureus, Klebsiella pneumoniae*). In particular, strains of *S. aureus*, *P. aeruginosa*, and *K. pneumoniae* were the most sensitive to the effects of CeO_2NPs compared to other bacteria (Dar et al., 2022).

The fundamental basis for the antibacterial behavior of nano-CeO_2 is two types of interaction with the bacterial cell membrane:

1) direct — binding of CeO_2NPs to membrane receptors and induction of an increase in reactive oxygen species (ROS) levels during the reversible conversion of Ce^{3+} to Ce^{4+} (Zhang et al., 2019);

2) indirect — transfer of ROS from the intercellular space to the bacterial cell membrane (Zholobak et al., 2016).

Nano-CeO_2 enhanced the antimicrobial activity of cell-free supernatants of probiotic bacilli against *P. vulgaris* 72, *E. coli* 028, *S. aureus* 209 and *C. albicans* 690. It is known that *P. vulgaris* and *E. coli* are similar in structure and do not form exopolysaccharide protectors. Accordingly, this facilitates the entry of CeO_2NPs into the cell, which inactivates important enzymes during complexation reactions, leading to a delay in pathogen reproduction or death (Reshma et al., 2017; Zhuo et al., 2021). The mechanism of inhibition of *S. aureus* growth by nano-CeO_2 is

related to the ability of nanoparticles to initiate the interaction of ROS with the thiol groups of peptidoglycan (Putri et al., 2021). Regarding *C. albicans*, the adsorption of CeO_2NPs on the cell wall of the fungus blocks the enzyme system of the microorganism (Babenko et al., 2012).

It can be postulated that the augmented antimicrobial activity of cell-free supernatants of probiotic strains IMV B-7142 and IMV B-7143 in the presence of CeO_2NPs is a consequence of the synergistic interaction of these nanoparticles with bacterial metabolites.

The binding of silver nanoparticles (AgNPS) to bacteriocin (BC), a synthesis product of *Lactobacillus paracasei*, enhanced the antibacterial activity of the compound against antibiotic-resistant bacteria. The action mechanism of the AgNPS/BC complex on pathogens included an impaired cell membrane permeability, elevated levels of ROS, and the destruction of proteins and DNA (Gomaa, 2019).

The lack of notable differences in the inhibition of *P. aeruginosa* ATCC 27959 growth by cell-free supernatants of the IMV B-7142 and IMV B-7143 strains that were obtained following the cultivation of bacteria with and without CeO_2NPs may be attributed to the pathogen's capacity to synthesize a complex of exopolysaccharides under stressful conditions. These biopolymers prevent the adsorption of nanoparticles on the cell membrane surface and their penetration into the cytoplasm, in conjunction with antibiotic compounds (Sahli et al., 2022; Chung et al., 2023).

The ability of nano-CeO_2 to nanomodulate biological processes, in particular, those based on antioxidant mechanisms, has been proven (Zholobak et al., 2014; Nelson et al., 2016), since the antioxidant system of any living organism is the first to respond to the effects of various physical and chemical factors.

The most sensitive and rapid method for determining the antiradical activity of biological material is the reaction with DPPH·, which allows for determining the ability of antioxidants to inactivate free radicals (Shimada et al., 1992). It was found that the antiradical activity of cell-free extracts of the probiotic bacteria *B. amyloliquefaciens* ssp. *plantarum* increased after their cultivation with nano-CeO_2 (0.01 mM) for 24 — 48 h, in particular, for strain IMV B-7142 — by 2.7 — 25.1%, and for strain IMV B-7143 — by 1.9 — 6.1%, compared to the control. It should be assumed that CeO_2NPs can enhance the antiradical activity of biological material by forming an electron pair on the nitrogen atom in DPPH·.

It is important to study the ability of antioxidant systems of living organisms to eliminate superoxide anion radicals ($O_2^{\bullet-}$), which can reduce Fe^{3+} ions to Fe^{2+} ions, which are the initiators of hydroxyl radical formation (Martemucci et al., 2022). It has been shown that during the cultivation of probiotic bacteria with nano-CeO_2, high $O_2^{\bullet-}$ interception activity was characteristic only for the cell-free supernatant of the IMV B-7143 strain.

The superoxide anion radical is formed as a result of normal cellular metabolism and acts as a signaling molecule. However, the background level of $O_2^{\bullet-}$ can increase rapidly due to the disruption of various biochemical processes in the cell. The toxic effects of excess $O_2^{\bullet-}$are usually controlled and reduced by the activity of superoxide dismutase , which deactivates $O_2^{\bullet-}$by the donor-acceptor pathway, converting this ROS to H_2O_2 and O_2 (Zheng et al., 2023). A similar mechanism of superoxide anion radical deactivation is characteristic of nano-CeO_2 (Reed et al., 2014). Therefore, CeO_2NPs can be considered nanoenzymes (Corsi et al., 2023).

The cultivation of the IMV B-7142 and IMV B-7143 strains in the nutrient medium with CeO_2NPs had a stimulating effect on the reducing power of their cell-free supernatants. The ability of CeO_2NPs to enhance the studied antioxidant index is related to the structural properties of nanoparticles. In particular, nano-CeO_2 can act as a catalyst for both oxidation and reduction simultaneously (Khan et al., 2023).

The hydroxyl radical (·OH) is one of the strongest oxidants of biologically active molecules. There are two ways in which living systems remove hydroxyl radicals:

1) blocking hydroxyl radical initiation reactions with the help of antioxidant enzymes (superoxide dismutase, glutathione peroxidase, and catalase);

2) interruption of the hydroxyl radical chain reaction with the help of non-enzymatic antioxidants (Lipinski, 2011; Jomova et al., 2023).

Dowdling with co-authors (Dowding et al., 2013) showed that CeO_2NPs are active hydroxyl radical scavengers. Other scientists (Xue et al., 2012; Zhang et al., 2014) demonstrated that CeO_2NPs can protect DNA from damage caused by ·OH attack.

The results indicate that nano-CeO_2 enhances the ability of cell-free supernatants of probiotic bacilli to scavenge hydroxyl radicals. It can be postulated that CeO_2NPs can form bioconjugates with antioxidant compounds present in cell-free bacterial supernatants. It is known that nanoparticles bind to antioxidant molecules and can transport them into living cells, thereby enhancing the antioxidant and antiradical properties of these compounds (Sharpe et al., 2011).

Conclusions. The results of the study on the effect of CeO_2NPs on the probiotic activity of *B. amyloliquefaciens* ssp. *plantarum* IMV B-7142 and IMV B-7143 demonstrate a stimulating effect on their growth and antimicrobial activity. The concentration-dependent effect of nanoparticles on the growth activity of bacilli was revealed: the lower the concentration, the higher the number of bacterial cells. The optimal concentration of nano-CeO_2 was found to be 0.01 mM.

The addition of nanoceria to the nutrient medium of *B. amyloliquefaciens* ssp. *plantarum* IMV B-7142 resulted in a higher level of antimicrobial activity against the test cultures *P. vulgaris* 72, *E. coli* 028, *S. aureus* 209, and *C. albicans* 690, compared to the IMV B-7142 strain. It was demonstrated that the antimicrobial effect was more pronounced after 48 hours of cultivation. This indicates that nano-CeO_2 can bind to antimicrobial metabolites of probiotics, which active synthesis on the second day of bacterial cultivation.

The antioxidant potential of probiotic strains *B. amyloliquefaciens* ssp. *plantarum* IMV B-7142 and IMV B-7143 increased under the influence of CeO_2NPs but to a different extent. This is probably due to the difference in the composition and properties of antioxidant metabolites (amino acids, polysaccharides, phenolic compounds) synthesized by bacteria, which can react with nanomaterials and inhibit reactive oxygen species.

It can be concluded that nano-CeO_2, due to its stimulatory effect on the probiotic activity and antioxidant potential of *B. amyloliquefaciens* ssp. *plantarum* IMV B-7142 and IMV B-7143, has the potential to be a promising component (prebiotic) for the creation of a biocomposite preparation for the prevention and treatment of infections. Further studies on experimental animals are required to confirm the therapeutic and prophylactic efficacy of the proposed complex preparation, which is based on nanoceria and probiotic bacillus strains.

Despite the compelling evidence of the role of probiotics in combating pathogens and the pressing necessity to devise novel strategies to combat antibiotic resistance, research in this field is still in its infancy and requires further development and discussion.

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ВПЛИВ НАНОЦЕРІЮ НА ПРОБІОТИЧНІ ВЛАСТИВОСТІ ШТАМІВ *BACILLUS AMYLOLIQUEFACIENS* SSP. *PLANTARUM*

Новим підходом до терапевтичного посилення пробіотиків є їх інтеграція з характерними (мають позитивний ефект на живі організми) наночастками. **Мета.** Дослідити вплив наноцерію (нано-CeO_2) на ріст пробіотичних бацил, а також їхню антимікробну активність і антиоксидантний потенціал для розробки біокомпозитного препарату для профілактики та лікування інфекцій. **Методи.** Кількість життєздатних бактеріальних клітин визначали методом десятикратного розведення. Антимікробну активність пробіотиків оцінювали за допомогою диск-дифузії, лункових дифузійних тестів та вимірювання зон пригнічення росту тест-культур (*Proteus vulgaris, Escherichia coli, Staphylococcus aureus, Candida albicans, Pseudomonas aeruginosa*). Спектрофотометричними методами досліджено антиоксидантний потенціал безклітинних супернатантів пробіотичних бактерій. **Результати.** Ростова активність пробіотичних штамів *Bacillus amyloliquefaciens* ssp. *plantarum* IMB B-7142 та *Bacillus amyloliquefaciens* ssp. *plantarum* IMB B-7143 досягала свого максимуму при вирощуванні з 0,01 мМ нано-CeO_2. Ця концентрація була визначена як оптимальна. При дослідженні антимікробної активності пробіотичних бацил під впливом нано-CeO_2 виявлено його стимулюючу дію, але різного ступеня для кожного з двох досліджуваних штамів. *B. amyloliquefaciens* ssp. *plantarum* IMB B-7142 пригнічував ріст умовно-патогенних тест-культур активніше, ніж штам IMB B-7143. Антиоксидантний потенціал безклітинних супернатантів досліджуваних бактерій був посилений нано-CeO_2, хоча й різною мірою. Зокрема, порівняно з контролем, помітно підвищувалась активність поглинання гідроксильних радикалів. **Висновки.** Отже, нано-CeO_2, завдяки його стимулюючій дії на фізіологічну та біохімічну активність *B. amyloliquefaciens* ssp. *plantarum* IMB B-7142 та IMB B-7143, може бути перспективним компонентом біокомпозитного препарату.

Ключові слова: *Bacillus amyloliquefaciens ssp. plantarum, наночастинки діоксиду церію, антимікробна активність, антиоксидантний потенціал.*