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EFFECT OF BIOGENIC AGNPs ON THE VIABILITY AND ADHESIVE PROPERTIES OF BACTERIA

Aim. *The study demonstrates the efficacy of silver nanoparticles (AgNPs), produced via green synthesis using L. acidophilus UKM B-2691, with regard to the metabolic activity and adhesive properties of Gram-positive and Gram-negative bacteria.* ***Methods.*** *Gram-positive strains (Staphylococcus aureus ATCC 25923, Bacillus subtilis ATCC 6633) and Gram-negative strains (Escherichia coli ATCC 25922, Pseudomonas aeruginosa PA01) were used as test cultures to evaluate the biological activity of biogenic AgNPs within a nanoparticle concentration range of 0.01—10 mM.* ***Results.*** *A correlation was observed between the reduction in metabolic activity and the suppression of adhesion. Particular attention is given to the discovered effect of significant adhesion inhibition at sublethal nanoparticle concentrations, where bacteria maintain high level of metabolic activity but lose the ability to colonize surfaces. It was found that Gram-positive test cultures exhibit higher sensitivity to AgNPs within a concentration range of 0.01—10 mM compared to Gram-negative strains. The results indicate the ability of biogenic AgNPs to inhibit early stages of bacterial metabolic activity and significantly reduce their adhesive properties, leading to the suppression of mature biofilm formation.* ***Conclusions.*** *These findings offer promising perspectives for the development of next-generation antimicrobial agents with high biocompatibility.*

Keywords: *nanosilver, adhesion, antibacterial properties, bacteria*

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Introduction

Nanoparticles (NPs) are materials with dimension ranging from 1 to 100 nm. Their unique characteristics, which distinguish them from their larger counterparts, are due to their extremely small size and high surface area-to-volume ratio. These features impart unique physical, chemical, and biological properties to NPs, opening broad opportunities for their application across various fields [1]. Among different types of metal-based nanoparticles, AgNPs are among the most widely used in modern biotechnological applications. AgNPs have gained wide recognition as highly effective antimicrobial agents capable of affecting a wide spectrum of pathogenic microorganisms [2]. Silver nanoparticles exhibit strong antibacterial activity (*Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Salmonella typhi, Pseudomonas aeruginosa*, *etc.*) and antifungal activity (*Alternaria alternata, Sclerotinia sclerotiorum, Macrophomina phaseolina, Rhizoctonia solani, Botrytis cinerea*, *etc.*) [3—6]. Their functionality further enhancds both optical characteristics, in particular localized surface plasmon resonance (LSPR), and electronic and catalytic properties, which are essential for applications for biosensing and biomedicine [7].

Biologically synthesized AgNPs are characterized by a natural surface coating formed by molecules of natural origin. This provides them with a high ability to easily interact with both bacterial cells and bactericides, which in turn enhances the overall antimicrobial activity of such nanoparticles [8]. Among AgNPs biosynthesis methods, special attention is given to approaches that involve reduction and stabilization of nanoparticles using biological agents, such as plants, algae, bacteria, or fungi.

The literature sources describe three phases of AgNPs interaction with bacterial cells [9]. The first one involves the interaction of silver nanoparticles (AgNPs) and silver ions with the bacterial cell membrane. This causes increased permeability and structural damage, resulting in membrane disruption and leakage of cytoplasmic contents.

The second mechanism is associated with the penetration of AgNPs and silver ions into the bacteria. Once inside the cell, they destabilize key biomolecules such as proteins, lipids, and DNA, leading to the disruptions in essential processes, including protein synthesis, respiratory chain function, ATP formation, gene transcription, and DNA replication.

The third mechanism involves oxidation and gradual corrosion of AgNPs with subsequent formation of silver ions in biological media. These ions disrupt cellular systems, including disulfide bond formation, metabolic processes, and iron homeostasis maintenance. This causes excessive production of reactive oxygen species (ROS), further increasing bacterial membrane permeability and enhancing antimicrobial effect [10, 11].

Alongside the investigation of the antibacterial mechanisms of AgNPs, it has been established that the characteristics such as size, charge, and surface properties of AgNPs significantly influence the viability of microorganisms. The size and charge of nanoparticals, along with their stability, are critical factors determining the antibacterial efficacy of AgNPs [12].

Overcoming bacterial resistance, particularly through the disruption and prevention of biofilm formation, remains a key challenge in modern medicine. In biomedicine, this is of particular importance for the development of antibacterial coatings for implants and catheters, where the primary mechanism is the inhibition of microbial adhesion to material surfaces. A promising direction also lies in the application of AgNPs in combination therapy as synergistic agents with traditional antibiotics, which can enhance their efficacy and facilitate overcoming bacterial resistance.

Thus, the investigation on the antibacterial potential of silver nanoparticles obtained via ecofriendly "green" synthesis, along with the determination of their dose-dependent effects on the metabolic activity and architecture of bacterial populations, with a specific focus on establishing a direct dependence between the degree of metabolic inhibition and the loss of adhesive potential, which

justifies the use of biogenic AgNPs as specific anti-adhesive agents, will provide new perspectives for the development of next-generation antimicrobial agents with high biocompatibility.

Materials and Methods

Bacterial test cultures

To investigate antibacterial properties, the following test cultures were used: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* PA01, and *Bacillus subtilis* ATCC 6633. These were obtained from the Ukrainian Collection of Microorganisms at the D.K. Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine.

Media for cultivation of bacterial test cultures

Nutrient agar (NA, Conda, Spain) was used for obtaining and maintaining test cultures, while Nutrient broth (NB, Conda, Spain) was used for biomass accumulation and testing antimicrobial properties. NB composition, g/L: peptic digest of animal tissue — 5.0, meat extract — 1.5, yeast extract — 1.5, sodium chloride — 5.0, distilled water — 1000. NA additionally contained 13 g/L agar-agar. Medium pH — 7.2—7.6. Media were sterilised in an autoclave at 121 °C for 15 min.

Characteristics of silver nanoparticles

Silver nanoparticles (AgNPs) were synthesized earlier by us via green synthesis in the Laboratory of the Department of Leather and Fur Biotechnology at KNUTD using metabolites of *L. acidophilus* UCM B-2691 [13]. According to dynamic light scattering (DLS) data, the average hydrodynamic diameter is 29 nm, the polydispersity index (PdI) is 0.242, and the Intercept is 0.87. The UV-visible absorption spectrum shows an intense surface plasmon resonance peak at 410 nm, confirming the formation of silver nanoparticles. The silver nanoparticles are brown in color, spherical in shape, and 29 nm in size. The initial silver concentration was 100 mM. For testing antimicrobial properties, nanosilver concentrations ranging from 0.005 mM to 10 mM were used.

Determination of test culture sensitivity to antimicrobial agents using resazurin

Antimicrobial activity was determined using the resazurin (Merck, Germany) reduction assay [14]. Test cultures grown in nutrient broth (NB) were inoculated at 10% (v/v) into a 96-well plate containing NB medium with AgNPs at concentrations from 0.005 to 10 mM. All experiments were performed in triplicate. Sterile distilled water (10%) was added to the control. Plates were incubated in a thermostat at 37 °C for 24 h. After incubation, 10 μL of sodium resazurin dye was added to each well and kept for up to 2 h at 37 °C. Spectrophotometric measurements were taken at 570 nm using a 620 nm reference wavelength on a HIPPO 96 universal UV microplate reader (Biosan, Lithuania). Next, the number of viable cells in the experimental samples was calculated as a percentage relative to the control which was set at 100%.

Determination of anti-adhesive properties

Evaluation of the adhesive potential of bacteria on abiotic surfaces is essential for studying microbial pathogenicity and biofilm formation [12]. One of the most common approaches is staining surface-adhered biomass with crystal violet, which binds to polysaccharide-protein components of the cell wall and biofilm matrix.

The method is based on the ability of bacterial cells to form an initial adhesive layer on an inert material (polystyrene). After incubation of test cultures, an adhered fraction forms, reflecting the initial stage of the biofilm process. Crystal violet (Merck KGaA, Germany) acts as a non-selective cationic dye that interacts with negatively charged cell structures and the extracellular matrix.

Grown test cultures *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *P. aeruginosa* PA01 and *B. subtilis* ATCC 6633 in nutrient broth (NB) were added to a 96-well plate containing NB medium with AgNPs at concentrations 0.005—10 mM. Sterile distilled water was added to the control. The plates were sealed and placed in a thermostat for cultivation at a temperature of 37 °C for 24 h. After incubation, the non-adhered fraction was removed per biosafety requirements, and gentian violet dye (100 μL) was added to each well, forming a dye complex with bacterial cell surface structures and matrix components. Binding intensity was considered an indirect indicator of adhesive potential. Wells were incubated for 5 min at 20 °C. The dye was then removed, and the wells were washed twice with deionized water. After drying the washed wells, 96% ethanol was added. Samples were incubated for 1 h and measured spectrophotometrically at 595 nm using a HIPPO 96 UV reader (Biosan, Lithuania). Data are expressed as percentages relative to the untreated control (taken as 100%) and presented as mean ± SD (n = 3 independent experiments). Error bars indicate standard deviation. Viable cells in experimental samples are expressed as percentages relative to the untreated control (taken as 100%) and presented as mean ± SD (n = 3 independent experiments). Error bars indicate standard deviation.

Statistical processing of results

Statistical analysis of data was performed using Microsoft Office Excel 2021. Spectrophotometrically obtained data were processed using the median. The control was set to 100%, and all other data were evaluated relative to each control.

Statistical analysis included calculation of mean values, standard deviation (SD), and Spearman's rank correlation coefficient (ρ) to assess the relationship between metabolic activity and adhesive ability of bacterial cells after AgNPs treatment. Correlation strength was interpreted according to the obtained ρ values, and statistical significance was accepted at $p < 0.05$.

Results and Discussion

Effect of nanosilver on metabolic activity and adhesive properties of ***Staphylococcus aureus*** *ATCC 25923*

The results, presented in Fig. 1, demonstrate that AgNPs exhibit a pronounced dose-dependent inhibitory effect on the metabolic activity (Fig. 1*a*) and adhesive properties (Fig. 1*b*) of *Staphylococcus aureus* ATCC 25923. In the control sample (C), bacterial metabolic activity was taken as 100%, characterizing normal bacterial metabolism. At nanosilver concentrations of 5 and 10 mM, the activity was completely inhibited, indicating a complete loss of metabolic activity due to the action of nanoparticles. Even at 1 mM, metabolic activity was only 8%, indicating near-complete blockage of the metabolic processes in bacterial cells. Further reduction in AgNPs concentration was accompanied by gradual partial recovery of metabolic activity. The fluctuations in metabolic activity at low concentrations may be related to the adaptive mechanisms of *S. aureus* ATCC 25923 cells or incomplete cell envelope permeability to nanoparticles at lower doses.

The data on adhesive properties (Fig. 1b) show that nanosilver also reduces significantly the ability of *S. aureus* ATCC 25923 cells to attach to surfaces and form biofilms. At AgNPs concentrations of 5 and 10 mM, adhesion was completely absent, indicating total destruction or inactivation of surface structures responsible for attachment. At 1 mM, adhesiveness remains at a level of only 17%, which correlates with the low level of metabolic activity and indicates an almost complete loss of functional cell integrity. Further reduction of silver nanoparticle concentration (0.005—0.5 mM) led to partial recovery of adhesion; however, even at the lowest tested concentration, the number of attached cells did not exceed 11%. This indicates that minimal AgNPs doses affect the adhesive capacity of *S. aureus* ATCC 25923, likely due to the disruption of synthesis of exopolysaccharides and adhesion proteins required for biofilm formation.

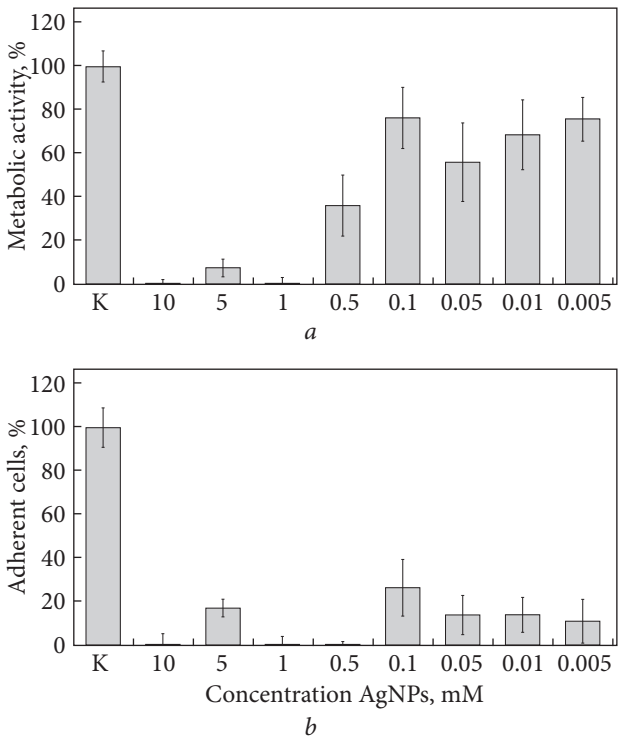

Fig. 1. Effect of different AgNPs concentrations on metabolic activity (*a*) and adhesive properties (*b*) of *Staphylococcus aureus* ATCC 25923

The results obtained indicate that silver nanoparticles exhibit antimicrobial activity against *S. aureus* ATCC 25923 by suppressing both metabolic activity and adhesive properties of the cells. At AgNPs concentrations of 1—10 mM, near-complete inhibition of bacterial viability was observed, accompanied by complete absence or minimal levels (17%) of adhesive properties. At the same time, at AgNPs concentrations of 0.005—0.5 mM, a discrepancy between viability and adhesion processes was detected. Even under conditions of partial metabolic activity recovery (36—76%), nanoparticles continued to effectively block adhesion, which did not exceed 27%. This mismatch between preserved metabolism and inhibited adhesion suggests a targeted effect of AgNPs on surface adhesins (protein molecules) located on cell surfaces; however, this requires further investigation at the molecular level. As a result of the interaction, *S. aureus* ATCC 25923 cells lose the ability to initiate biofilm formation and remain in a planktonic state, maintaining viability at a sublethal level.

Correlation analysis confirmed a statistically significant positive association between metabolic activity and adhesion (Spearman's $\rho = 0.71$, $p = 0.031$), suggesting that suppression of bacterial metabolism by AgNPs directly affects the ability of cells to adhere to surfaces.

Effect of nanosilver on metabolic activity and adhesive properties of ***Bacillus subtilis*** *ATCC 6633*

The results presented in Fig. 2 show a pronounced dose-dependent effect of silver nanoparticles on the metabolic activity (Fig. 2*a*) and adhesive properties (Fig. 2*b*) *of Bacillus subtilis* ATCC 6633.

High AgNPs concentrations (1, 5, and 10 mM) caused sharp suppression of metabolism. When the nanosilver concentration was reduced to 0.5 mM, the cell survival increased to 15%, and further decrease in AgNPs concentration was accompanied by substantial recovery of metabolic processes: 84% (0.1 mM), 75% (0.05 mM), 69% (0.01 mM), and 83% (0.005 mM). This dependence indicates the reduction of toxic effect of AgNPs at lower concentrations and the activation of adaptive survival mechanisms in *B. subtilis* ATCC 6633 aimed at restoring the cellular functions.

A similar pattern was observed for adhesive properties of *B. subtilis* ATCC 6633 (Fig. 2*b*). With increasing AgNPs concentration, the number of adhered cells decreased; specifically, the percentage of adhered cells was 8% at 0.005 mM, 17% at 0.01 mM, 19% at 0.05 mM, 9% at 0.1 mM, and 4% at 0.5 mM, whereas at 1, 5, and 10 mM adhesion was absent (0—1%). Despite the recovery of metabolic activity to levels above 80% within the 0.1—0.005 mM concentration range, the cell adhesiveness remained low (up to 20%). This indicates that even at low doses, silver nanoparticles affect bacterial surface structures, in particular by disrupting adhesin proteins and polysaccharides that mediate cell attachment to surfaces.

A strong positive correlation was observed between metabolic activity and adhesive ability of *B. subtilis* ATCC 6633 treated with AgNPs (Spearman's $\rho = 0.87$, $p = 0.003$). This indicates that suppression of bacterial metabolic processes was accompanied by a significant reduction in cell adhesion.

Thus, silver nanoparticles exert a dual dose-dependent effect on *B. subtilis* ATCC 6633. At high concentrations complete suppression of metabolic activity occurs, while at low concentrations partial metabolism recovery occurs without restoration of adhesive properties. This may be associated with prolonged disruption of cell surface structures, indicating a persistent anti-attachment effect of AgNPs even as their toxicity decreases.

Effect of nanosilver on metabolic activity and adhesive properties of ***Escherichia coli*** *ATCC 25922*

According to the results of studying the effect of different silver nanoparticle concentrations on the metabolic activity of *Escherichia coli* ATCC 25922 (Fig. 3*a*), low AgNPs concentrations exhibited a stimulatory effect, whereas high concentrations caused the growth inhibition and bacterial cell death. Presumably, low nanosilver concentrations (0.01—1 mM) induced a moderate oxidative stress, which activated the cellular defense mechanisms and resulted in increased metabolic activity. Due to specific features of *E. coli* ATCC 25922 metabolism, a pronounced stimulatory effect of silver nanoparticles was observed. The most pronounced increase in metabolic activity occurred at 0.5 mM AgNPs, exceeding the control value by more than twofold (209%). A concentration of 1 mM AgNPs also promoted increased cellular activity relative to the control (133%). Concentrations of 0.1 mM, 0.05 mM, and 0.01 mM produced a moderate increase in metabolic activity (102%, 108%, and 119%, respectively), indicating a weak stimulatory effect.

The results obtained may be partially explained by structural features of the outer membrane of

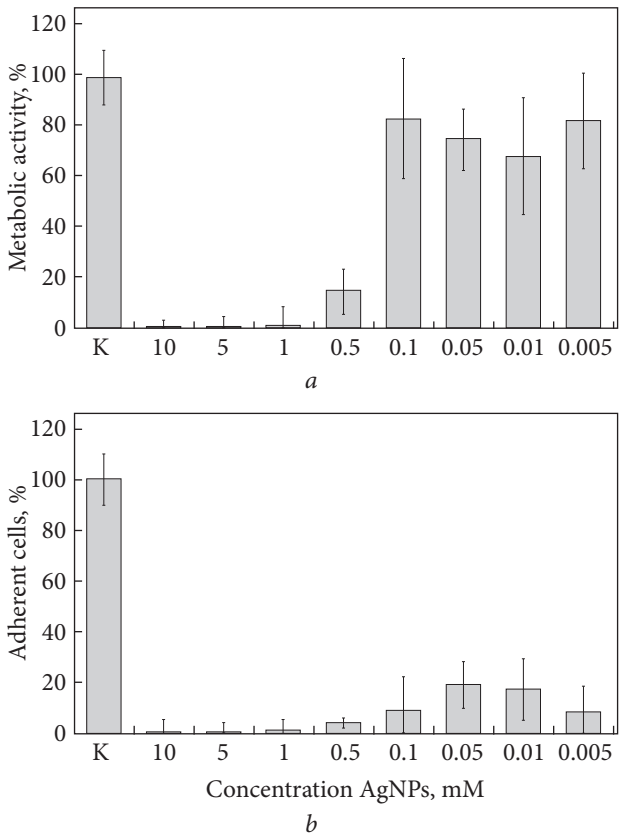

Fig. 2. Effect of various AgNPs concentrations on metabolic activity (*a*) and adhesive properties (*b*) of *Bacillus subtilis* ATCC 6633

Gram-negative bacteria. The presence of a substantial amount of lipopolysaccharides reduces cellular permeability to silver nanoparticles at low concentrations. In contrast, high AgNPs concentrations (5 and 10 mM) exceeded the compensatory capacity of the cell, leading to disruption of the electron transport chain and decreased bacterial metabolic activity. Compared with the control, metabolic activity of *E. coli* ATCC 25922 in the presence of AgNPs at 5 and 10 mM was 12% and 0%, respectively, indicating complete suppression of metabolism. A slight decrease in metabolic activity was also observed at 0.05 mM (81%), suggesting that the antibacterial properties of silver nanoparticles do not follow strictly linear relationship with concentration.

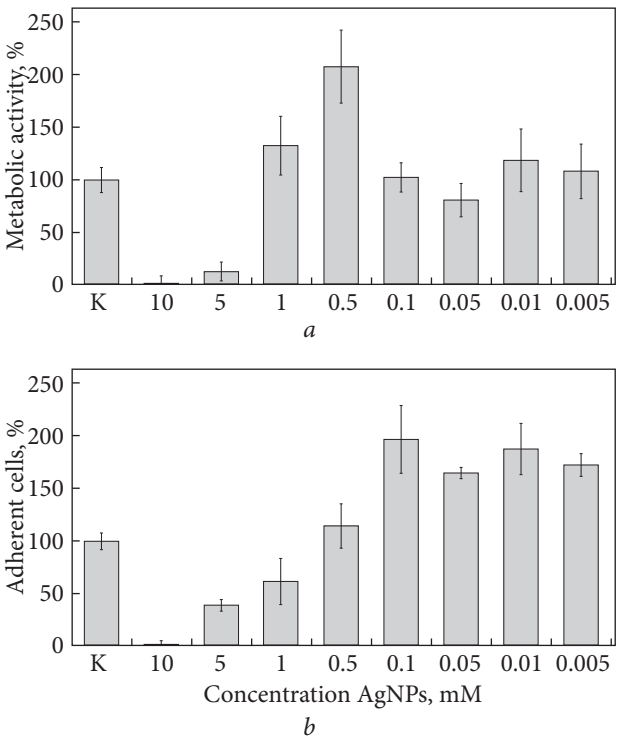

Fig. 3. Effect of various AgNPs concentrations on metabolic activity (*a*) and adhesive properties (*b*) of *Escherichia coli* ATCC 25922

Analysis of the effect of silver nanoparticles on the adhesive properties of *E. coli* ATCC 25922 (Fig. 3*b*) showed that the response depends on AgNPs concentration and may manifest as either stimulation or inhibition of cell attachment. At higher nanoparticle concentrations (1, 5, and 10 mM), a pronounced anti-adhesive effect was observed. Specifically, at 10 mM adhesion was completely suppressed (0%), whereas at 5 mM the number of adhered cells decreased to 39%. At 1 mM, the adhesion level increased but remained below control values (63%). In contrast, reducing AgNPs concentration within the 0.5—0.05 mM range was accompanied by a stimulatory effect on adhesion. The maximum adhesive activity was observed at 0.1 mM, where the number of attached cells reached 197% relative to the control. A concentration of 0.5 mM had a moderate effect (115%), whereas at 0.05 mM, 0.01 mM, and 0.005 mM adhesive properties increased to 165%, 188%, and 173%, respectively. Presumably, the relatively weak anti-adhesive action at low nanoparticle concentrations is associated with the presence of the outer membrane, which in Gram-negative bacteria performs a barrier function, protecting the cell not only from toxic agents but also from disruption of the synthesis of adhesive components.

A notable observation concerns the marginal stimulation of metabolic activity in *E. coli* ATCC 25922 at minimum AgNPs concentrations (0.005—0.5 mM), with a peak increase reaching 209% relative to the control at 0.5 mM. This phenomenon can be characterized as hormesis, a biphasic dose-response relationship where low levels of a stressor elicit a stimulatory or compensatory adaptive response [15]. In the context of silver nanoparticles, this metabolic activation is likely driven by a controlled increase in the production of reactive oxygen species (ROS), which at sublethal concentrations function as secondary messengers. This triggers the activation of the SoxRS and OxyR regulons, which not only upregulate antioxidant enzymes but also induce a transient shift in metabolic flux to satisfy the increased energy demands for cellular repair and ion efflux mechanisms [16]. Thus, the observed stimulation reflects an adaptive oxidative stress response, where the bacterial population temporarily enhances its metabolic output to counteract potential silver-induced damage.

The structural features of the Gram-negative cell envelope play a key role in this response. The outer membrane of *E. coli* acts as a permeability barrier, limiting the penetration of AgNPs and released Ag^+ ions into the cytoplasm. This shielding effect substantially reduces acute toxicity at low doses, thereby promoting adaptive survival responses and protecting adhesive structures from direct impact. Consequently, *E. coli* ATCC 25922 is characterized by relatively low sensitivity to silver nanoparticles at concentrations below 1 mM. A pronounced inhibitory effect on both metabolic activity and adhesion is observed only at concentrations of 1 mM and above, where the compensatory capacity of the

cell is overwhelmed, leading to the transition from stimulation to established inhibitory action [17].

These complexities are further reflected in the statistical analysis. Correlation analysis for *E. coli* ATCC 25922 revealed a weak positive but statistically insignificant relationship between metabolic activity and adhesive ability after AgNPs treatment (Spearman's $\rho = 0.33$, $p = 0.381$). This lack of significance is explained by the pronounced hormetic effect; the significant metabolic «flare-up» induced by adaptive stress-response mechanisms does not result in a proportional increase in adhesion. This dissociation confirms that under subinhibitory conditions, the metabolic turnover is redirected toward survival and homeostasis rather than the maintenance of adhesive properties, further justifying the potential of biogenic AgNPs as specific anti-adhesive agents even in strains with high natural resistance.

Effect of nanosilver on metabolic activity and adhesive properties of ***Pseudomonas aeruginosa*** *PA01*

According to the effect of various silver nanoparticle concentrations on the metabolic activity (Fig. 4*a*) of *Pseudomonas aeruginosa* PA01 cells, this culture is quite resistant to nanosilver. The metabolic activity reduction occurs only at AgNPs concentrations above 0.5 mM. Compared to the control, concentrations of 10 mM, 5 mM, 1 mM, and 0.5 mM led to complete or nearly complete metabolism inhibition (0%, 2%, 0%, and 3%, respectively), indicating a strong toxic effect of the nanoparticles. At 0.1 mM, metabolic activity increased to 74%; however, it remained below the control level. Nanosilver concentrations below 0.05 mM had virtually no effect on cellular metabolic activity (0.05 mM — 103%; 0.01 mM and 0.005 mM — 101%), which may be associated with an adaptive bacterial response or stimulation of metabolic processes in response to the low, stress-inducing doses of nanoparticles.

Under nanosilver exposure, the adhesive properties of *P. aeruginosa* PA01 (Fig. 4*b*) were significantly lower compared with the control. The use of AgNPs

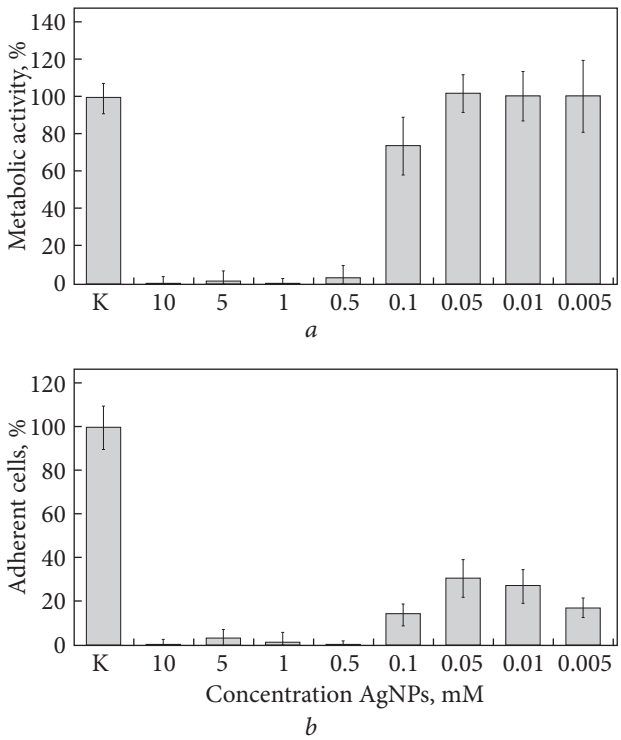

Fig. 4. Effect of various AgNPs concentrations on metabolic activity (*a*) and adhesive properties (*b*) of *Pseudomonas aeruginosa* PA01

concentrations from 0.5 to 10 mM led to a complete loss of adhesive properties in *P. aeruginosa* PA01, indicating substantial inhibition of the cells' ability to attach to a surface. In contrast, at AgNPs concentrations from 0.005 to 0.1 mM, a partial recovery of the adhesion capacity of *P. aeruginosa* PA01 cells was observed; however, the proportion of adhered cells did not exceed 31%.

Correlation analysis for *P. aeruginosa* PA01 revealed a strong positive and statistically significant relationship between metabolic activity and adhesive ability after AgNPs treatment (Spearman's $\rho = 0.83$, $p = 0.006$). This indicates that suppression of bacterial metabolic processes was accompanied by a marked reduction in cell adhesion.

Thus, AgNPs concentrations ranging from 0.5 mM to 10 mM exert an inhibitory effect on both the metabolism and the adhesive properties of *P. aeruginosa* PA01. Within the range of 0.1 mM to 0.005 mM, the

toxic effect is markedly reduced: metabolic activity approaches the control level, whereas adhesive properties recover only to approximately 30%. Therefore, silver nanoparticles demonstrate a pronounced dose-dependent antibacterial effect on *P. aeruginosa* PA01.

The scientific novelty of this study lies in establishing a direct correlation between the suppression of metabolic activity and the loss of adhesive properties in bacterial cells under the influence of biogenic silver nanoparticles (AgNPs) synthesized using *L. acidophilus* UCM B-2691. A key element of this novelty is the identification of a significant reduction in bacterial adhesion even at sublethal AgNPs concentrations. Consequently, these biogenic nanoparticles function as specific anti-adhesive agents capable of blocking surface colonization without necessitating total population eradication, thereby minimizing selective pressure and mitigating the risk of antimicrobial resistance.

To study the mechanisms involved, it is essential to distinguish the biological activity of AgNPs from that of ionic silver (Ag^+). While the release of ions contributes to overall metabolic inhibition [19], the pronounced anti-adhesive effect observed at sublethal doses suggests a nanoparticle-specific interaction [20]. This process is mediated by the organic capping layer derived from the *L. acidophilus* UCM B-2691 supernatant, which modulates the interface between the nanoparticle and the bacterial cell wall. This biogenic shell facilitates the blocking of adhesins more effectively than free Ag^+ ions could at equivalent concentrations [21].

In addition, the scientific novelty is emphasized by the determination of species-specific sensitivity governed by the cell envelope architecture. The absence of an outer membrane in Gram-positive bacteria (*S. aureus* ATCC 25923, *B. subtilis* ATCC 6633) facilitates a more direct and intensive interaction of AgNPs with the cytoplasmic membrane compared to Gram-negative strains (*E. coli* ATCC 25922, *P. aeruginosa* PA01), where the lipopolysaccharide layer acts as a partial permeability barrier. Taken together, these results provide a comprehensive understanding of the multi-level antimicrobial impact of biogenic nanostructures, establishing a foundation for their application in anti-biofilm materials; however, further cytotoxicity and biocompatibility studies are required to confirm their clinical safety.

Conclusion

Based on the results of the studies, it has been established that biogenic silver nanoparticles (AgNPs) exert a pronounced dose-dependent effect on the metabolic activity and adhesive properties of Gram-positive and Gram-negative bacteria, supported by an experimentally demonstrated correlation.

The results show that AgNPs produced via green synthesis demonstrate a protent multi-level antimicrobial effect, which includes the suppression of metabolism and the disruption of adhesive structures, thereby reducing the capacity to form a biofilm. The higher sensitivity observed in Gram-positive species (*S. aureus*, *B. subtilis*) is attributed to the structural features of their cell envelope; specifically, despite its significant thickness, the porous nature of the peptidoglycan layer allows for easier nanoparticle penetration compared to the protective outer membrane of Gram-negative strains (*E. coli*, *P. aeruginosa*), which acts as a robust permeability barrier. Furthermore, the efficacy of biogenic AgNPs at sublethal concentrations as targeted anti-adhesive agents is confirmed, allowing for the blocking of surface colonization at early stages without the need for complete eradication of the population — a mechanism that effectively reduces selective pressure and limits the emergence of antimicrobial resistance. These findings underscore a promising potential of using biogenic AgNPs as contact-action antimicrobial agents with broad-spectrum activity, although further investigations on their cytotoxicity and biocompatibility remain essential to establish their safety for clinical applications.

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ВПЛИВ БІОГЕННИХ AGNPS НА ЖИТТЄЗДАТНІСТЬ
ТА АДГЕЗИВНІ ВЛАСТИВОСТІ БАКТЕРІЙ

Мета. Показати ефективність впливу наночасток срібла (AgNPs) отриманих зеленим синтезом за допомогою *L. acidophilus* УКМ В-2691 на метаболічну активність та адгезивні властивості грам-позитивних і грам-негативних бактерій. ***Методи.*** Для оцінки біологічної активності біогенних AgNPs як тест-культури було використано грам-позитивні штами (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633) та грам-негативні штами (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* PA01) у діапазоні концентрацій наночастинок 0,01—10 мМ. ***Результати.*** Показано кореляцію між зниженням метаболітичної активності та блокуванням адгезії. Особлива увага приділялася виявленому ефекту значного пригнічення адгезії при сублетальних концентраціях наночастинок, де бактерії зберігають високий рівень метаболічної активності, але втрачають здатність колонізувати поверхні. Виявлено, що грам-позитивні тест-культури виявляють вищу чутливість до впливу AgNPs у діапазоні концентрацій 0,01—10 мМ порівняно з грам-негативними штамами. Отримані результати показують здатність біогенних AgNPs інгібувати ранні етапи метаболічної активності бактерій та суттєвого знижувати їх адгезивні властивості, що призводить до блокування формування зрілих біоплівок. Висновоки. Отримані результати відкривають перспективи для розробки антимікробних засобів нового покоління з високою біосумісністю.

***Ключові слова*:** наносрібло, адгезія, антибактеріальні властивості, бактерії.