



RESEARCH ARTICLE

Temperature-Dependent Biosynthesis and Comparative Characterization of Silver Nanoparticles by *Stenotrophomonas maltophilia* With Multifunctional Activities

Esra Erkoç^{1a} , Seval Taliboğlu^{1b} , Filiz Korkmaz^{2a} , Hakan Eskizengin^{1c} , Fadime Kıran^{1d} , Nur Koçberber Kılıç^{1e*}

^{1a}Department of Biology, Faculty of Science,
Ankara University, Ankara, Türkiye

²Biophysics Laboratory, Faculty of Engineering,
Atılım University, Ankara, Türkiye

^{1a}: <https://orcid.org/0000-0003-2457-2735>

^{1b}: <https://orcid.org/0009-0009-8837-9572>

^{1c}: <https://orcid.org/0000-0003-1709-7497>

^{1d}: <https://orcid.org/0000-0002-4536-2959>

^{1e}: <https://orcid.org/0000-0003-2668-3789>

^{2a}: <https://orcid.org/0000-0003-3512-3521>

*Corresponding Author:
Nur Koçberber Kılıç
E-mail: nrkilic@ankara.edu.tr

Received: January 23, 2026

Revised: March 04, 2026

Accepted: April 30, 2026

ABSTRACT: Biogenic AgNP production at room temperature (20 ± 2 °C) and 37 °C were investigated using *Stenotrophomonas maltophilia*. Transmission electron microscopy showed average particle sizes of 9.1 ± 2.6 nm at room temperature and 6.8 ± 1.8 nm at 37 °C. ATR-FTIR analysis indicated that the nanoparticles were associated with biomolecules, mainly proteins and lipids, suggesting a role in stabilization. The synthesized AgNPs exhibited antibacterial activity against *Staphylococcus aureus* ATCC 29213 (17 ± 0.2 mm inhibition zone) and 84% antibiofilm activity against *Pseudomonas aeruginosa*. Photocatalytic results showed 57% removal of methylene blue within 20 minutes. Cytotoxicity assays revealed a dose-dependent response, with the strongest effect in A549 cells (IC_{50} : 6.54 µg/mL). All experiments were performed in at least biological triplicates, and statistical significance was evaluated where applicable ($p < 0.05$). These findings highlight the multifunctional nature of biogenic AgNPs and support their relevance for antimicrobial and environmental applications within the scope of in vitro conditions.

Keywords: *Stenotrophomonas maltophilia*, green synthesis, AgNP, antimicrobial, photocatalytic, cytotoxicity

INTRODUCTION

Among nanomaterials, silver nanoparticles (AgNPs) are one of the metal nanoparticles that have been extensively investigated for various purposes. Silver nanoparticles have become quite useful due to their physicochemical and biological properties, large surface-to-volume ratio, ease of modification with different types of ligands, antimicrobial effects, effective cytotoxicity against cancer cells, etc.

Different methods can be used in the production of AgNPs, including physical, chemical, and biological [1]. Pyrolysis, tube furnace (evaporation-condensation), laser ablation, gamma-irradiation, and microwave processing are among the physical methods. Chemical methods include the Sol-Gel method, co-precipitation, chemical vapor deposition, etc. In these methods, heat application and, the use of toxic and secondary, tertiary chemicals creates harmful effects on humans and the environment. The chemicals used in particles synthesized through chemical methods along with the toxicity of the particles, and their reactivity, and the by-products formed could create negative effects on humans and the environment. On the other hand, while the physical methods

might offer a safer alternative approach for nanoparticle production, they are not preferred due to the excessive use of the equipment and high cost [2].

Considering these issues, green synthesis stands out as an eco-friendly approach to produce nanoparticles. Also, aggregation and stability of the produced nanoparticles by this green synthesis are the significant factors in determining its suitability for practical applications. The rapid production of nanoparticles produced by this method, especially bacteria-assisted green synthesis, along with the production of ready-to-use nanoparticles and the natural presence of substances that provide stabilization of the nanoparticle makes this method even preferable [2-6].

In the production of AgNPs, bacteria are quite interesting among different types of organisms. For example, one study reported an absorption peak at 420 nm in UV-Vis spectra of AgNPs synthesized using *S. maltophilia* (S54), which falls within the characteristic range expected for classical AgNPs [7]. They synthesized spherical AgNPs with sizes of 15-50 nm. In another study stated that they synthesized AgNPs with sizes of 2-20 nm using recombinant chitinase enzyme obtained from *S. maltophilia* [8]. AgNPs with sizes ranging from 40–60

nm and a heterogeneous distribution were synthesized using bacterial supernatant of *Stenotrophomonas* sp. [9].

In another report, showed that nanoparticles with an average size range of 12-27 nm obtained from the *S. maltophilia* NS-24 had antimicrobial effects on pathogenic bacteria such as *Escherichia coli*, *Enterococcus faecalis*, *Streptococcus pneumoniae*, and *Staphylococcus aureus* [10]. In a study keratinase from *S. maltophilia* R13 was used as a reducing agent for the synthesis of AgNPs. They showed that AgNPs had the highest antimicrobial effect against *Klebsiella pneumonia* with an inhibition zone of 15.6 mm [11].

Synthesis of AgNPs can be carried out under different temperature conditions and it is known that temperature conditions influence the shapes and sizes of nanoparticles. For example, a study carried out the synthesis of AgNPs at room temperature (20 °C), yielding spherical particles with sizes ranging from 1.9 to 14.1 nm [5]. In another study, it was shown that the tested bacteria synthesized particles with sizes of 30-75 nm at 27 °C [12]. A study showed the synthesis of AgNPs by bacteria at 22 °C [13]. In another study conducted at 25 °C, 35 °C, and 40 °C to produce AgNPs, it was observed that the optimal temperature was 35 °C [14].

AgNPs have the potential to be used for a wide range of purposes. One of the potential applications of AgNPs is the removal of bacterial biofilm. Bacteria synthesize biofilm to protect themselves from adverse conditions and provide biofilm formation. Nanoparticles, especially AgNPs are used as an environmentally friendly approach to combat biofilm producing drug-resistant bacteria [15, 16]. In addition to their use in biofilm removal, their high photocatalytic activity is one of the most notable properties that broadens their potential applications. When previous studies were examined, it was seen that biogenic AgNP synthesis was performed using different bacteria, however, studies determining the photocatalytic activities of these nanoparticles were limited. In a study conducted to investigate the potential of AgNPs obtained from *Lactacaseibacillus rhamnosus* in photocatalytic applications; they observed an increase in dye removal over time in the presence of 20 mg AgNPs in the medium containing 10 mg/L MB under visible light [17]. When the photocatalytic effect of AgNPs synthesized intra- and extracellularly by *Bacillus cereus* was examined, researchers stated that extracellularly synthesized nanoparticles had more photocatalytic activity. In that study, at 50 mg/L MB 100% dye removal was observed in 80 minutes with the application of extracellularly synthesized AgNPs [18].

In addition to their antibiofilm and high photocatalytic properties, AgNPs are considered important for cancer treatment due to their strong cytotoxic effects [19]. Several in vitro studies using AgNPs have demonstrated their strong anticancer efficacies in a variety of cancer cells [20, 21].

AgNPs with varying sizes, shapes, and zeta-potentials may exhibit distinct anticancer effects, owing to differences in membrane permeability and electrostatic interactions with the cell membrane. Therefore, it is crucial to assess the anticancer effect of each distinct formulation of AgNP individually, due to their varying physicochemical properties.

Despite extensive studies on biogenic AgNPs, the relationship between bacterial metal resistance traits and their influence on nanoparticle formation, biomolecular capping, and resulting multifunctional bioactivity remains insufficiently explored. In this study, *Stenotrophomonas maltophilia*, a previously reported heavy metal-resistant bacterium with high extracellular polymeric substance (EPS) production capacity, was selected. Compared to many conventional bacterial systems, its strong metal tolerance and enhanced biomolecule secretion profile provide a favorable environment for efficient extracellular nanoparticle synthesis and stabilization. These characteristics make *S. maltophilia* a promising biological platform for controlled and stable AgNP production.

The synthesized biogenic AgNPs were characterized using UV-Vis spectroscopy, transmission electron microscopy (TEM), and ATR-FTIR spectroscopy. In addition, their multifunctional properties were systematically evaluated, including antibacterial, antibiofilm, photocatalytic, and cytotoxic activities.

This study highlights the relationship between bacterial metal resistance traits, biomolecular capping, and the multifunctional performance of AgNPs. The integrated evaluation of synthesis conditions, structural characteristics, and functional activities provides a comprehensive assessment of AgNPs produced by metal-resistant *S. maltophilia*. In particular, the observed photocatalytic efficiency broadens the functional scope of these biogenic nanoparticles, suggesting their potential relevance not only for antimicrobial applications but also for nanoremediation under in vitro conditions.

MATERIALS AND METHODS

AgNP production by *S. maltophilia*

S. maltophilia was obtained from the culture collection of Bioremediation Research Laboratory, Department of Biology, Faculty of Science, Ankara University (Ankara, Türkiye) [22]. For AgNP production, *S. maltophilia* was grown in 100 mL NB medium for 3 days. At the end of the incubation period, centrifugation was performed at 6000 rpm for 10 minutes to remove the biomass (Hermle, Germany). The supernatant (5 mL) was transferred to a 1 mM AgNO₃ (pH 7) solution prepared in 45 mL in brown bottles. This mixture was incubated statically at room temperature (20 ± 2 °C) and 37

°C at 150 rpm with shaking for 24, 48, and 72 hours [23]. The synthesized AgNPs were centrifuged at 10.000 rpm for 15 minutes to separate the supernatant from the pellet. The resulting pellets were dried overnight at 80 °C in glass petri dishes; the obtained powder was then recovered and stored in a cool, dry conditions.

AgNP Characterization

UV-Vis spectrophotometric analysis

Optical characterization of biogenic AgNPs was performed using a UV-Vis spectrophotometer (BioDrop, UK). Absorption spectra of the synthesized AgNP solution were scanned in the range of 300–600 nm.

TEM (Transmission Electron Microscope)

The size, shape, arrangement, elemental composition, and purity of the synthesized AgNPs were characterized using a transmission electron microscope (TEM, JEOL JEM 100 CXII, Tokyo, Japan). The diameters of the produced nanoparticles were measured using the ImageJ software (version 1.53n) based on at least 100 measurement points.

Attenuated Total Reflection Fourier transform Infrared Spectroscopy (ATR-FTIR) analysis

Five microliters from each sample were used for FTIR spectroscopy measurements. A spectrometer (Thermo-Scientific, Nicolet 6700) equipped with attenuated total reflection (ATR, ConcentratIR2, Harrick) accessory with ten internal reflections was utilized. Samples were first dried by a gentle stream N₂ gas for 15 min to remove excess water. Silver nanoparticles, in powder form, obtained as described above, were directly placed on the ATR diamond. The absorbance spectra were then recorded by collecting and averaging 120 scans at a resolution of 4/cm. Spectra were ATR-corrected using the instrument software OMNIC (version 8.2.388) and used for the visuals presented in this study without further pre-processing. Difference spectra were obtained by subtracting two area-normalized absorbance spectra from each other. The area area-normalization eliminates the concentration differences to a large extent. A subtraction factor is determined interactively using the display window until the resulting spectrum shows positive and negative peaks distributed around the zero line.

Determination of Antibacterial Activity

Disk diffusion method was used to determine the antibacterial activity of the AgNPs [24]. For this purpose, *E. coli* ATCC 10536, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 29213, *Bacillus subtilis* ATCC 6633 were used as standard bacterial strains. These strains were obtained from

the culture collection of Bioremediation Research Laboratory, Department of Biology, Faculty of Science, Ankara University (Ankara, Türkiye). After overnight growth in liquid medium, the bacterial suspensions were standardized to the 0.5 McFarland turbidity and inoculated into Nutrient Agar plates using sterile swabs. 40 µl AgNP (10 mg/mL) was applied to sterile discs placed in the inoculated petri dishes. The Petri dishes containing the discs were incubated at 37 °C for 24 hours. Discs loaded with antibiotic (streptomycin) were used as positive controls. The antibacterial activity of nanoparticles was evaluated by measuring the inhibition zones in millimetres.

Determination of Antibiofilm Activity

Dried AgNPs were used to investigate antibiofilm activity [25]. Biofilm-producing *P. aeruginosa* [26] grown in NB medium was incubated for 24 hours at 37 °C in a 96-well microplate containing increasing AgNPs concentrations, with a total volume of 200 µL. *P. aeruginosa* was obtained from the culture collection of Bioremediation Research Laboratory, Department of Biology, Faculty of Science, Ankara University (Ankara, Türkiye). At the end of the incubation period, the wells were washed with sterile distilled water and left to dry at room temperature. Then, fixation was performed using 100 µL of ethanol. After fixation, 200 µL of crystal violet solution [(0.1% (w/v))] was added to the wells. A washing step was then carried out to remove crystal violet from the wells. As the final step, 200 µL of 33% glacial acetic acid was added [27, 28] to the wells and the absorbance was measured at 590 nm using a microplate reader (BMG Labtech, Spectrostar Nano).

Determination of Photocatalytic Activity

A 24W xenon lamp was used as a light source to determine photocatalytic activity. 75 mL of distilled water containing 20 mg/L Methylene Blue (MB) was prepared in 150 mL Erlenmeyer flasks as the working solution. 25 mg AgNP was added to this medium and stirred in the dark for 30 minutes. The working solution that reached equilibrium was continuously stirred under a light source and experiments were carried out. Samples (2 mL) were collected at 10, 20, 40, 80, 160, and 320 min, then centrifuged (Hangzhou Yooning, Mini10K+ Mini Centrifuge) at 10.000 rpm for 10 min, and the supernatant was collected. The amount of dye remaining in the obtained supernatant was measured at 664 nm [29]. Control experiments (dye-only under light, dye + AgNPs in dark, and dye-only in dark) were performed to exclude adsorption and photolysis effects.

Determination of Cytotoxic Activity

Cytotoxic potential of the AgNPs was determined through MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium

bromide) analysis, which assessed the mitochondrial metabolic activities of the cells. Human breast cancer (MCF-7, ATCC HTB-22), non-small cell lung cancer (A549, ATCC CCL-185), and murine melanoma (B16-F10, ATCC CRL-6475) cell lines were used for this assay. The cells were grown in 75 cm² cell culture flasks with DMEM medium supplemented with 10% FBS (Sigma, USA) and 1% penicillin/streptomycin (Sigma, USA) and incubated at 37 °C in a humidified atmosphere with 5% CO₂. Upon reaching 70–80% confluence, cells were harvested using 0.25% trypsin-EDTA solution (Sigma, USA), then seeded into 96-well cell culture plates at a density of 5.000 cells per well and incubated overnight. After incubation, the cells were treated with different concentrations of AgNPs (0–100 µg/mL) for 24 hours. The cells cultured in DMEM medium without AgNPs were used as the negative control. Following the 24-hours AgNP treatment, the cells were incubated with MTT solution (5 mg/mL, Sigma, USA) for 4 hours. Then, the medium in the wells was removed, and formazan crystals at the bottom of the wells were dissolved with 100 µL dimethyl sulfoxide (Serva, USA). Cell viability was determined by measuring the absorbance of the formazan products at 570 nm using a microplate reader (BioTech, USA). Experiments were performed in triplicates, and the concentration of AgNPs that can induce 50% of cytotoxicity was determined

using GraphPad Prism software (version 9.0.2). All cell lines used in this study were authenticated, and routine testing confirmed that they were free of mycoplasma contamination.

All experiments were performed in at least biological triplicates with technical replicates where applicable. Data are presented as mean ± standard deviation (SD). Statistical significance was evaluated using one-way ANOVA followed by Tukey's multiple comparisons test, as indicated in the figure legends, with $p < 0.05$ considered statistically significant. Dose–response curves and IC₅₀ values were calculated using nonlinear regression analysis.

RESULTS AND DISCUSSION

In the experiments, when AgNP was produced, the brown color was observed, and the presence of particles was evaluated using UV-Vis spectroscopy. To determine the formation of extracellular AgNP, samples collected from the mixtures were monitored over the specified time interval by scanning UV-Vis absorption spectra in the range of 300–700 nm. The UV-vis analysis revealed that *S. maltophilia* produced AgNPs at a concentration of 3.1×10^{-8} M, as indicated by a prominent absorption peak at 428 nm in Fig. 1.

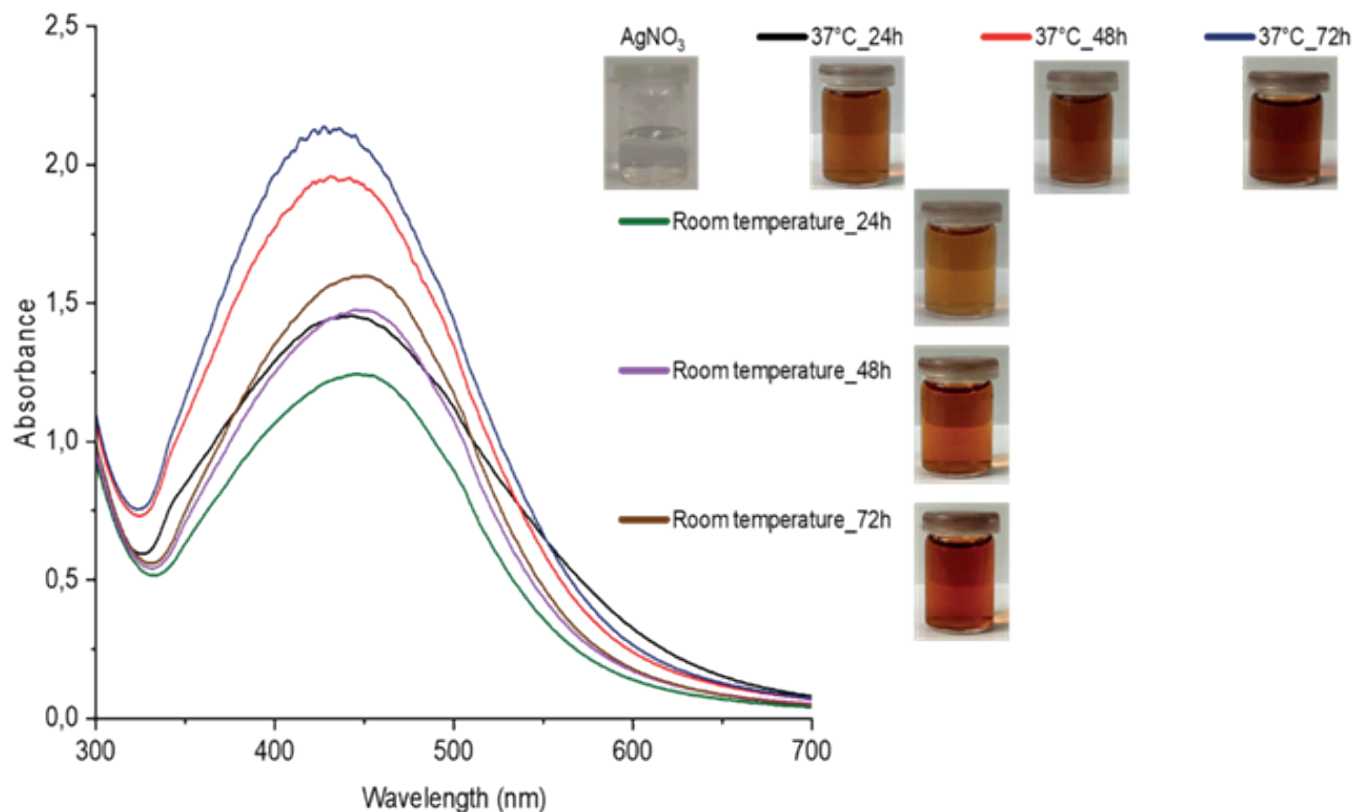


Fig. 1. UV-Visible spectra of AgNPs produced by *S. maltophilia* at room temperature (20 ± 2 °C) and 37 °C

The morphologies of AgNPs at room temperature (20 ± 2 °C) and 37 °C were determined by examining TEM micrograph images. The morphologies of AgNPs synthesized using cell-free supernatant were observed to be spherical. In 24-hour samples incubated at 37 °C, the particle size was found 6.8 ± 1.8 nm; while it was 7.6 ± 2.2 nm at the 48th hour and 8 ± 2.2 nm at the 72nd hour of incubation as shown in **Fig. 2 (a, c, e)**. The average particle size after 24, 48, and 72 hours of incubation at room temperature (20 ± 2 °C) was determined as 9.1 ± 2.6 , 9.4 ± 2.6 and 17.2 ± 3.2 nm, respectively as shown in **Fig. 2 (g, i, k)**. According to a previous study on AgNP production with *S. maltophilia*, the sizes of nanoparticles obtained from the supernatant of *S. maltophilia* strain NS-24 were in the range of 12.56 nm to 27.32 nm and the average size was 18.06 nm through TEM analysis [10]. In another study, AgNPs obtained with *Stenotrophomonas* sp. were mostly spherical and the average particle size was determined as 40-60 nm [9]. In the current study, it was observed that particle size increased as incubation time increased, regardless of temperature, consistent with the findings of, where particle size was shown to increase with incubation time [30]. Additionally, a study showed that the effectiveness of the investigated activities, such as antibacterial potential, increased with decreasing particle size [31]. Therefore, since the particles obtained at 37 °C in the 24-hour samples were smaller, the experiments were continued under these conditions.

The observed increase in nanoparticle size over time may be explained by several well-known mechanisms. One possible explanation is Ostwald ripening, where smaller particles dissolve and redeposit onto larger ones, leading to gradual growth in average particle size [32]. In addition, particle aggregation driven by reduced surface stabilization over time may contribute to size increase [33]. Furthermore, continued nucleation and growth processes during synthesis could also influence the final particle size distribution [34]. These mechanisms collectively may explain the time-dependent increase in particle size observed in this study.

The silver nanoparticles formed in the cell-free medium of *S. maltophilia* were analysed in dry form using ATR-FTIR spectroscopy. When the spectrum of the cell-free medium and the AgNPs formed in this medium are compared in **Fig. 3**, it is observed that most of the principal peaks exist in both, proving that the molecules in the cell-free medium stabilize and surround the AgNPs. The peaks at 1637/cm and 1542/cm assigned to amide I and II, respectively, confirm the existence of proteins as part of capping molecules. Two peaks at 1396 and 1333/cm are assigned to C-H bending of aldehydes and C-O stretching vibrations, respectively. The fingerprints of carbohydrates are seen in the range of 1200-900/cm. The peaks at 1143, 1085, 1044, and 979/cm are mainly due to C-O stretching modes and are clear fingerprints of polysaccharides [35, 36]. The peaks observed in the 3000-2800/cm range are attributed to C-H stretching modes. While C-H vibrations are a component of lipid spectra, the absence of the characteristic four-peak fingerprint (ascribed to specific CH₂ and CH₃ vibrational distributions) indicates that these signals originate from other organic structures. Therefore, the spectral profile does not support the presence of lipids, and these modes are attributed to non-lipid C-H containing species.

Although UV-Vis, FTIR, and TEM analyses were used for characterization, DLS and zeta potential measurements were not performed due to instrumental limitations. These analyses would provide additional insight into the colloidal stability and dispersion behavior of the nanoparticles.

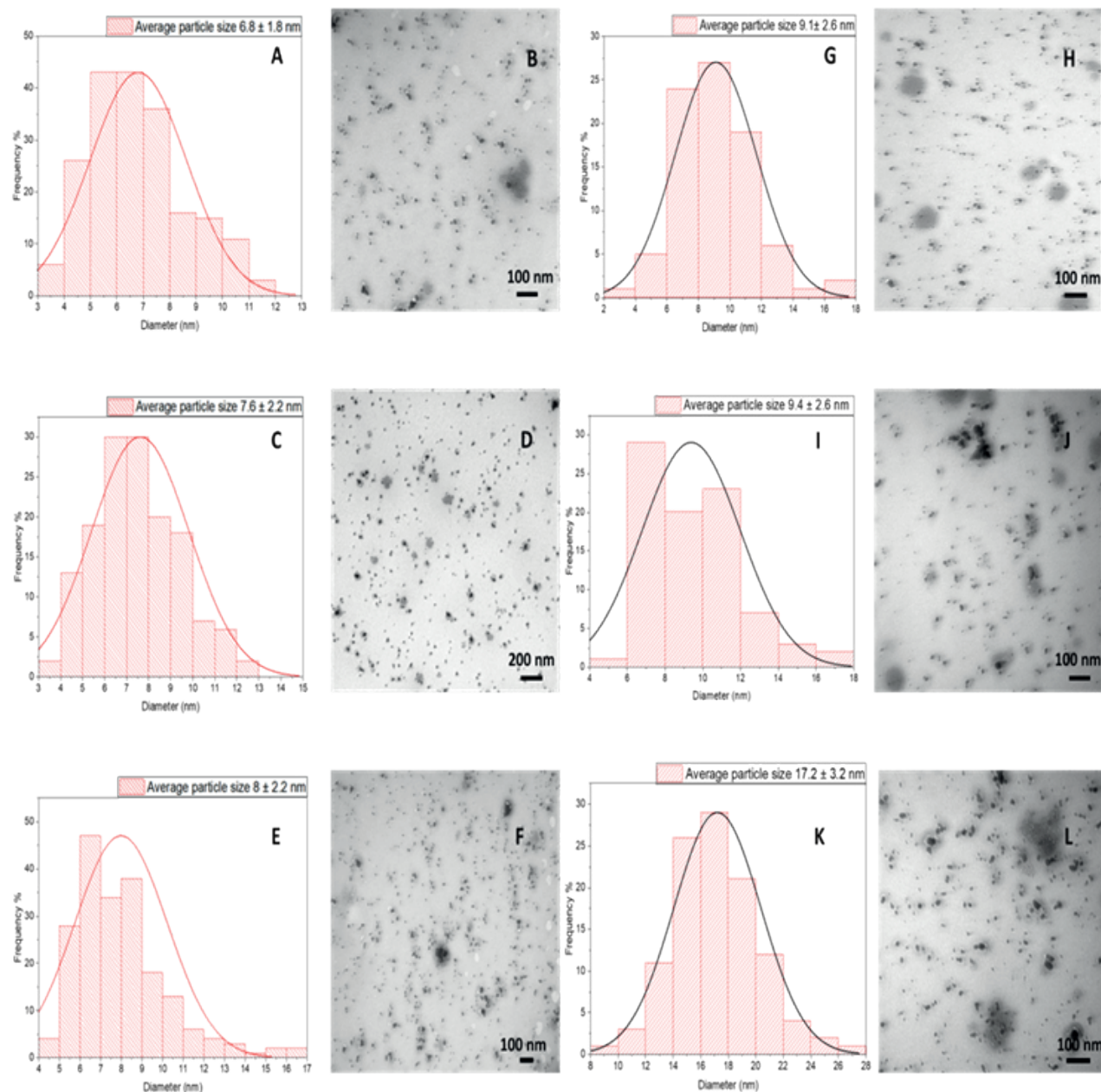


Fig. 2. TEM images and corresponding histograms of AgNPs synthesized by *S. maltophilia* at

37°C

- (A) Histogram after 24 hours of incubation
- (B) TEM image
- (C) Histogram after 48 hours of incubation
- (D) TEM image
- (E) Histogram after 72 hours of incubation

Room temperature (20 ± 2 °C)

- (G) Histogram after 24 hours of incubation
- (H) TEM image
- (I) Histogram after 48 hours of incubation
- (J) TEM image
- (K) Histogram after 72 hours of incubation

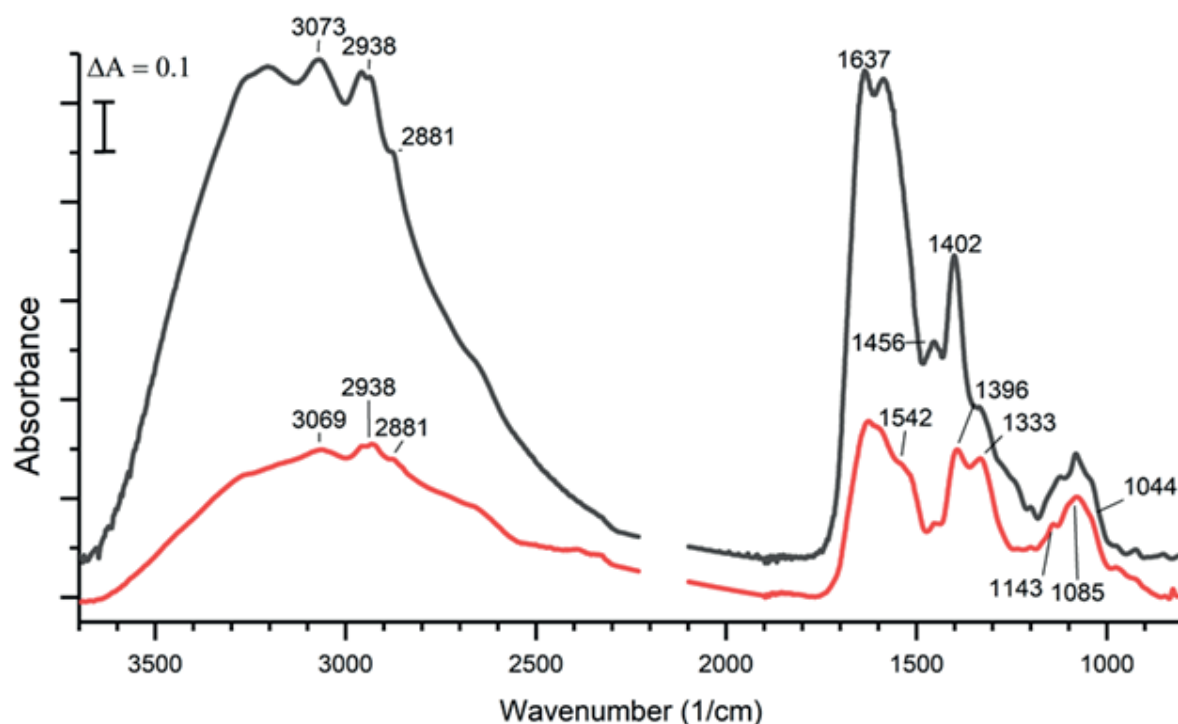


Fig. 3. Infrared spectrum of NB (black) and cell-free supernatant (red) of *S. maltophilia* grown in NB

Antibacterial activity of AgNPs

The antibacterial activity of AgNPs was tested against Gram-negative and Gram-positive bacteria. The highest antibacterial activity was found against *S. aureus* ATCC 29213 with a 17 ± 0.2 mm inhibition zone. The inhibition zone was measured as 15 mm against *E. coli* 0157:H7 ATCC 35150. AgNPs showed an antibacterial effect against *P. aeruginosa* ATCC 27853 with a 14 mm inhibition zone, while the inhibition zone against *B. subtilis* ATCC 6633 was 16 ± 0.2 mm showed that **Table 1** and **Fig. 4**. In this

study, the highest antibacterial activity of biogenic AgNPs was found against Gram-positive *S. aureus* ATCC 29213. The antibacterial activity in this study was assessed using the disk diffusion method as an initial screening approach. While this method provides comparative evidence of antimicrobial activity, quantitative parameters such as MIC and MBC, as well as comparative evaluations with AgNO_3 and bacterial supernatant, were not included. Therefore, the results reflect the antibacterial response under the tested conditions.

Table 1 Inhibition zone diameters (mm) of AgNPs synthesized by *S. maltophilia* showing antibacterial activity

Bacteria	Inhibition zone (mm) AgNP	Inhibition zone (mm) Antibiotic
<i>P. aeruginosa</i> ATCC 27853	14.0 ± 0	12.0 ± 0
<i>E. coli</i> 0157:H7 ATCC 35150	15.0 ± 0	19.0 ± 0.1
<i>S. aureus</i> ATCC 29213	17.0 ± 0.2	18.0 ± 0.2
<i>B. subtilis</i> ATCC 6633	16.0 ± 0.2	23.0 ± 0

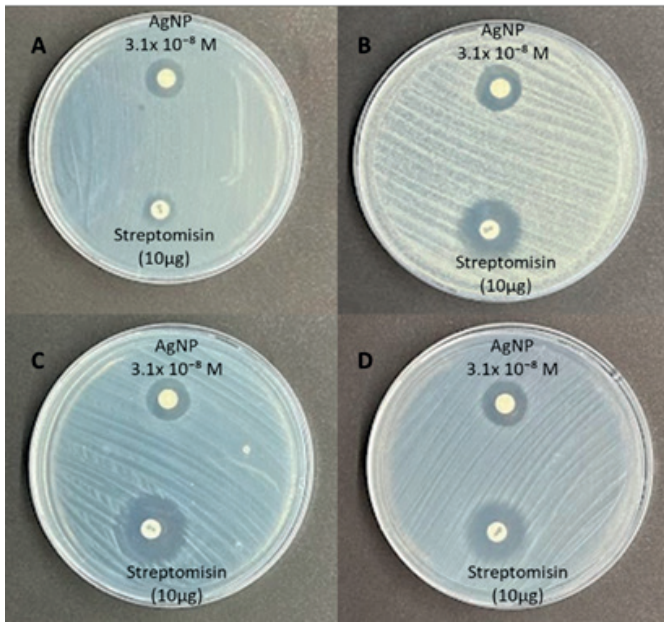


Fig. 4. Antibacterial activity of AgNPs against (A) *P. aeruginosa* ATCC 27853, (B) *E. coli* 0157:H7 ATCC 35150, (C) *S. aureus* ATCC 29213, (D) *B. subtilis* ATCC 6633

The antibacterial action of AgNPs operates through seven key mechanisms: Compromise of cell wall and membrane integrity, ribosomal denaturation and inhibition of protein synthesis, disruption of ATP production and energy metabolism, membrane damage induced by reactive oxygen species (ROS), inhibition of DNA replication, genetic disruption, membrane protein denaturation, and functional loss, and membrane perforation with increased permeability [37].

In previous studies, metal-based nanoparticles have been reported to have different antibacterial effects against Gram-negative and Gram-positive bacteria. Some researchers have shown that the antibacterial effect of AgNPs is greater on Gram-positives [38], while others have shown that it is more effective on Gram-negatives [37, 39]. Researchers attribute this situation to differences in the membrane structures of bacteria. In addition, Gram-negative strains are more sensitive than Gram-positive strains because positively charged AgNPs are attracted to the surface of negatively charged Gram-negative bacteria. The differences in the results of previous studies can be attributed to the dispersion medium, solubility, surface charge of NPs, geometric shape of NPs, and the nature of the tested bacterial strain. In the present study, higher antibacterial activity was observed against Gram-positive bacteria, which may be attributed to differences in cell wall composition. This trend is consistent with previously reported studies on biogenic AgNPs.

Antibiofilm activity of AgNPs

The antibiofilm effect of AgNPs obtained from *S. malto-philus* against *P. aeruginosa* was investigated. Increasing AgNP concentrations were adjusted between 0.5-500 µg/mL. Biofilm inhibition increased with the increase in nanoparticle concentration. The highest antibiofilm activity was found 84% at 500 µg/ml concentration. Antibiofilm activity was determined as 17%, 28%, and 54% against *P. aeruginosa* at 0.5, 5 and 50 µg/mL concentrations, respectively as shown in Fig 5. The antibiofilm activity in this study, assessed by crystal violet staining, reflects total biofilm biomass reduction; however, it does not allow differentiation between biofilm inhibition and eradication due to the absence of viability-based assays.

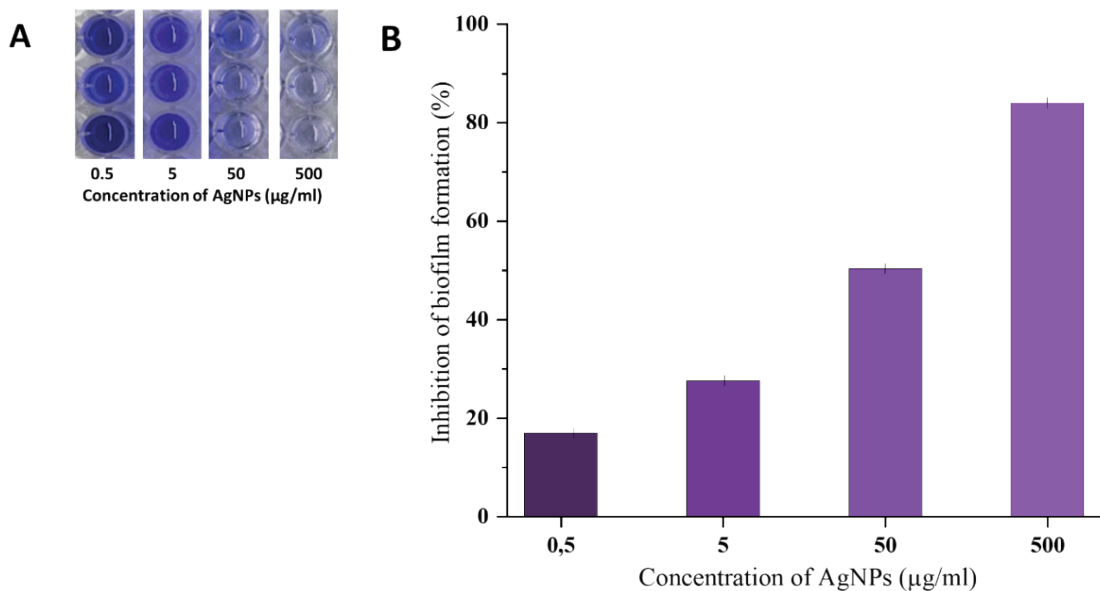


Fig. 5. The antibiofilm activity of AgNPs on *P. aeruginosa* (a) plate photo showing biofilm producer and antibiofilm activity of AgNPs (b) Antibiofilm activity (%) of AgNPs

Nanoparticles are recognized for their ability to inhibit biofilm formation. They possess the potential to interfere with the establishment and maturation of bacterial biofilms, which are a major concern in the treatment and prevention of bacterial infections. Consequently, nanoparticles are considered promising agents for disrupting biofilm integrity, thereby preventing bacterial adhesion, colonization, and proliferation.

To give a few examples of the antibiofilm activities of biogenically synthesized AgNPs, a study showed that green synthesized AgNPs with an average size of 10.8 nm were effective on biofilm-producing strains [40]. In the same study, they reported that biofilm inhibition percentages ranged from 2% to 89%. In another study, it was stated that green synthesized AgNPs had strong antibiofilm activity against *P. aeruginosa* 1149 [41]. AgNPs cause increased permeability of the bacterial cell membrane, induction of oxidative damage by reactive oxygen species; and damage to the cell membrane and intracellular proteins by interacting with phosphates in DNA. Thus, the biofilm production mechanism quorum-sensing is blocked, and the bacteria are prevented from producing biofilm [42]. The AgNPs tested in this study exhibited antibiofilm activity, which may be associated with the proposed mechanism described in this work.

Photocatalytic activity of AgNPs

The use of AgNPs in methylene blue (MB) removal was investigated under UV light. Photocatalytic activity of AgNPs and MB (20 mg/L) removal percentages are shown in **Fig. 6**. While the removal rate was observed as 56% in the first 10 minutes, the highest color removal was obtained 57% at the end of 20 minutes. Dye removal was nearly constant after contact time of 40 and 80 minutes. Dye removal slightly decreased with increasing contact time. Negligible dye degradation was observed under control conditions (dye under light without AgNPs and dye with AgNPs in dark), indicating that the observed dye removal occurred predominantly under photocatalytic conditions.

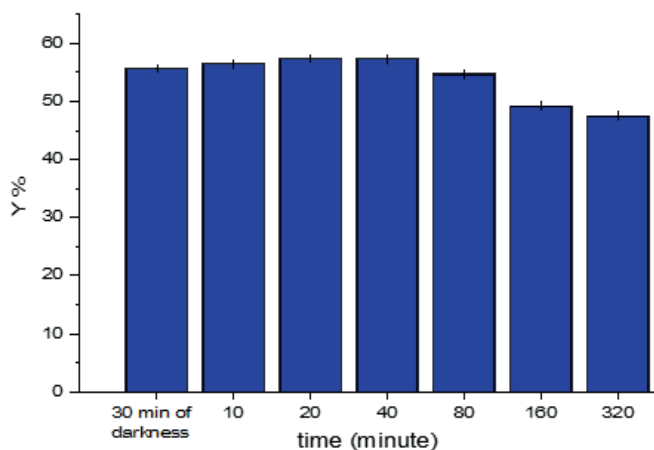


Fig. 6. Photocatalytic activity of AgNPs (C_0 : 20 mg/L; AgNP: 25 mg/75 mL; T: $20 \pm 2^\circ\text{C}$; Stirring speed: 200 rpm)

AgNPs are among the promising, efficient, and low-cost metal oxides used as photocatalysts for sustainable environmental remediation [43]. The narrow band gap makes AgNPs a suitable photocatalyst for the degradation of organic dyes under visible light irradiation [44, 45]. The stability of AgNPs is one of their interesting properties, which results in high photocatalytic activity. A study reported that a partial formation of metallic Ag occurred on the surface of Ag_2O during the photocatalysis of organic substances, which helped maintain the stability of the nanophotocatalyst [46].

Metallic Ag also participates in the photodegradation of organic pollutants, thereby enhancing the efficiency of the photocatalysis process. During the photo-induced process, the adsorption-desorption equilibrium was established in the absence of light to ensure that MB was fully adsorbed onto the catalyst surface. This suggests that the dye molecules underwent minimal adsorption or degradation under these conditions [47]. A key consideration in the removal of MB using AgNPs is whether the AgNPs are capped during synthesis. Coated AgNPs provide increased stability, preventing nanoparticle agglomeration and enhancing their efficiency in MB removal [48]. During AgNP synthesis, capping can occur either naturally or through artificial means, with the photocatalytic performance of the nanoparticles being influenced by the specific capping material used. For instance, previous studies have shown that the photocatalytic removal of MB using polyethylene glycol (PEG)-capped AgNPs reached 63.78% within 80 minutes [49], whereas uncapped AgNPs achieved complete degradation within the same duration [18]. However, it was also reported that the photocatalytic activities of intracellular and extracellular AgNPs differ, and a higher degree of capping may reduce the photocatalytic efficiency of AgNPs.

In this study, the highest removal percentage was observed within the first 20 minutes, after which the adsorption-desorption equilibrium between MB and AgNPs was likely reached. The synthesized biogenic AgNPs are capped with biomolecules, which may contribute to surface saturation during the initial stage. Following equilibrium, the contribution of photocatalytic activity to additional dye removal was limited, suggesting that adsorption plays a dominant role in the early phase of the process, while photo-induced enhancement remains relatively minor under the tested conditions.

Cytotoxic activity of AgNPs

The anticancer activity of biosynthesized AgNPs using *S. maltophilia* extracts was evaluated via MTT assay, which assesses mitochondrial activity as an indicator of cell viability. The results demonstrated a significant, dose-dependent reduction in cancer cell viability after 24 h of AgNPs treatment (**Fig. 7**).

In MCF-7 cells, treatment with 1 µg/mL AgNPs resulted in a 26% decrease in viability ($p < 0.0001$), while the highest concentration (100 µg/mL) led to a 98% reduction ($p < 0.0001$). A549 cells showed heightened sensitivity, with even the lowest dose (1 µg/mL) reducing cell viability by 53% ($p < 0.0001$). Although B16-F10 cells were less responsive compared to the other lines, cell viability decreased between 18% ($p < 0.001$) and 96% ($p < 0.0001$), depending on the concentra-

tion. Furthermore, IC_{50} values determined from the MTT assay were found as 25.84 µg/mL for MCF-7, 6.54 µg/mL for A549, and 30.07 µg/mL for B16-F10 cells. These findings suggest that AgNPs exhibit potent cytotoxic activity across different cancer cell lines, even at low concentrations. The inclusion of a normal mammalian cell line would further strengthen the evaluation of selective cytotoxicity.

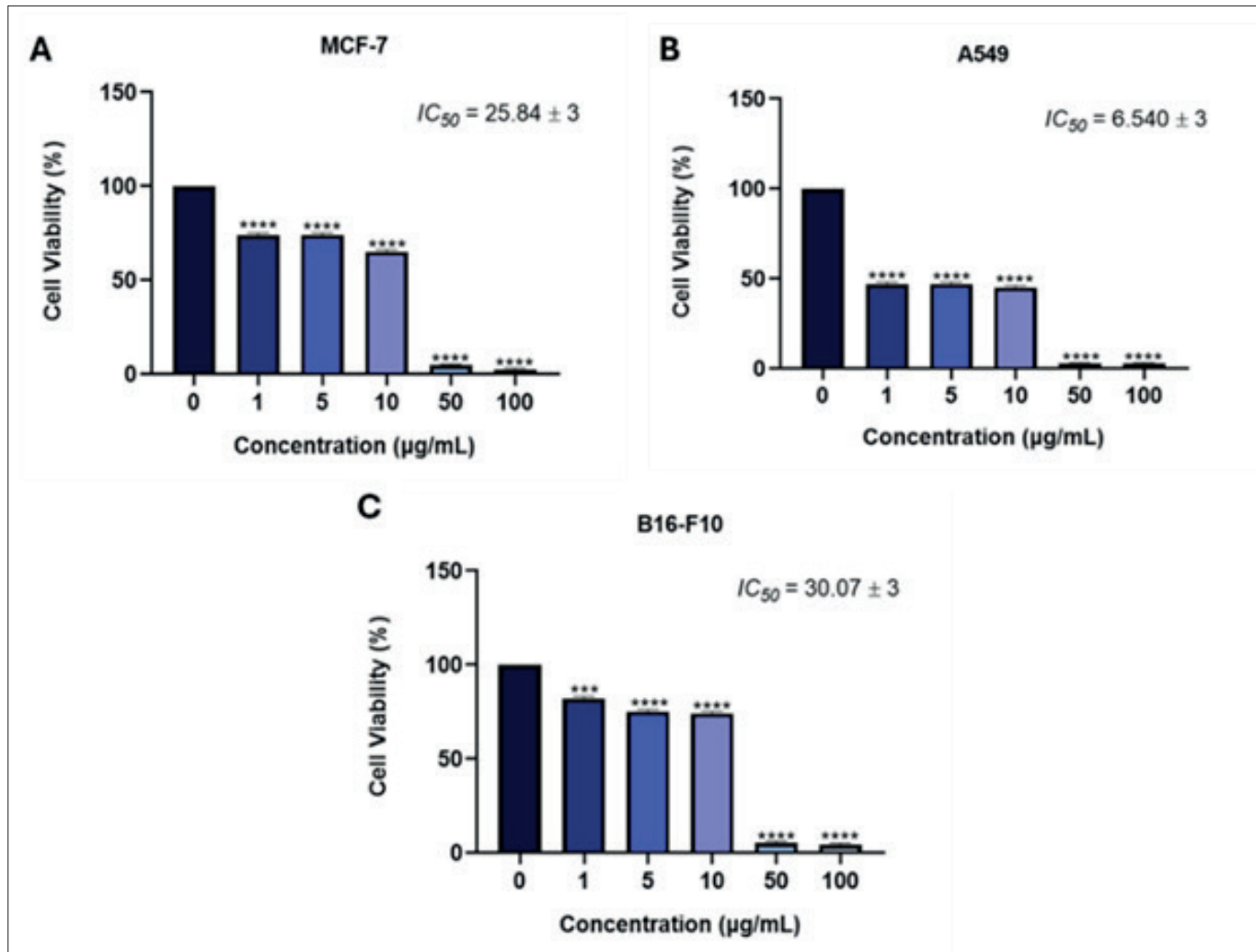


Fig. 7. Cell viability of MCF-7 (A), A549 (B), and B16-F10 (C), following 24-hour AgNPs treatment, as assessed by the MTT assay. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparisons test. Data are presented as mean $\pm$ SD. ns indicates non-significant differences; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ****: $p < 0.0001$.

Cancer is a major health concern, responsible for millions of deaths each year. Traditional treatment methods, such as radiotherapy, chemotherapy, and immunotherapy, often fail to provide adequate results, making the development of novel therapeutic approaches increasingly essential [50]. In recent years, nanotechnology has emerged as a promising field with the potential to revolutionize cancer treatment. Nanomateri-

als, particularly metal-based nanoparticles such as gold, silver, iron, zinc, titanium, nickel, copper, magnesium, and calcium, have attracted significant attention in cancer research due to their therapeutic potential [51]. AgNPs are particularly promising candidates for alternative cancer therapies, as they have demonstrated potent anticancer effects in both *in vitro* and *in vivo* studies [52-54].

In the present study, the cytotoxic effect of AgNPs synthesized via a green method using *S. maltophilia* as the bacterial source were evaluated on MCF-7, A549, and B16-F10 cancer cells, and their anticancer potential was evaluated. Similar findings have been reported in a previous study, where AgNPs derived from *S. maltophilia* showed significant cytotoxic activity against HeLa cervical cancer cells [55]. Additionally, a study observed a dose-dependent reduction in the viability of A549 lung cancer and HeLa cervical cancer cells after 48 hours of treatment of AgNPs derived from *E. coli* [56]. It was reported that AgNPs exert their anticancer effects by inducing apoptosis or necrosis through disruption of cell structures, as well as by generating reactive oxygen species (ROS) that cause DNA damage [57]. Moreover, AgNPs may enhance cancer cell death by activating specific enzymes and altering key signalling pathways and gene expression. A recent study demonstrated significant morphological changes in A549 lung cancer cells treated with AgNPs, as well as cell cycle arrest at the G2/M phase, leading to inhibition of cell proliferation [58]. Furthermore, AgNPs increased the expression of cytochrome c, initiator caspase 9, and executioner caspase 3, which are involved in the intrinsic apoptosis pathway, while suppressing the expression of anti-apoptotic genes in cancer cells. In another study, AgNPs synthesized from *Acinetobacter* sp. were shown to inhibit cell proliferation in HeLa cervical cancer cells due to accumulation in the subG1 phase [59]. These studies collectively highlight the significant potential of AgNPs, owing to their high cytotoxicity against various cancer cell lines.

CONCLUSION

In this study, extracellular synthesis of AgNPs using *S. maltophilia* was successfully achieved at both room temperature (20 ± 2 °C) and 37 °C, offering a potentially cost-effective production approach. The obtained nanoparticles were predominantly spherical, with smaller average sizes observed at 37 °C compared to room temperature based on TEM analysis. ATR-FTIR results indicated that biomolecules such as proteins and polysaccharides likely played a role in nanoparticle stabilization.

The synthesized AgNPs demonstrated antibacterial activity, particularly against *S. aureus* ATCC 29213, and exhibited notable antibiofilm effects under the tested conditions. In addition, moderate MB removal efficiency was observed within a short contact time. Cytotoxicity assays indicated a dose-dependent response in the tested cancer cell lines.

Overall, these findings suggest that biogenically synthesized AgNPs from *S. maltophilia* may have multifunctional properties relevant to antimicrobial and environmental applications. However, their potential and practical applicability should be interpreted considering the need for detailed mechanistic understanding, toxicity evaluation, and validation under in vivo conditions.

ACKNOWLEDGEMENT

This work was supported by the Ankara University Research Fund Project (FLO-2024-3358). Esra Erkoç acknowledges support from the TÜBİTAK (BİDEB) 2211-A National PhD Scholarship Program.”

REFERENCES

- [1] Iravani, S., Korbekandi, H., Mirmohammadi, S.V., Zolfaghari, B. (2014): Synthesis of silver nanoparticles: chemical, physical and biological methods. *Research in Pharmaceutical Sciences* 9: 385–406.
- [2] Hosny, S., Mohamed, L.Z., Ragab, M.S., Alomoush, Q.K., Abdalla, E.M., Aly, S.A. (2025): Nanomaterials in biomedical applications: opportunities and challenges—a review. *Chemical Papers* 1-22. <https://doi.org/10.1007/s11696-025-03937-5>
- [3] Sofi, M.A., Sunitha, S., Sofi, M.A., Pasha, S.K., Choi, D. (2022): An overview of antimicrobial and anticancer potential of silver nanoparticles. *Journal of King Saud University - Science* 34(2): 101791. <https://doi.org/10.1016/j.jksus.2021.101791>
- [4] Hussain, I., Singh, N.B., Singh, A., Singh, H., Singh, S.C. (2016): Green synthesis of nanoparticles and its potential application. *Biotechnology Letters* 38: 545-560. <https://doi.org/10.1007/s10529-015-2026-7>
- [5] Mageswari, A., Subramanian, P., Ravindran, V., Yesodharan, S., Bagavan, A., Rahuman, A.A. Karthikeyan, S., Gothandam, K.M. (2015): Synthesis and larvicidal activity of low-temperature stable silver nanoparticles from psychrotolerant *Pseudomonas mandelii*. *Environmental Science and Pollution Research* 22: 5383-5394. <https://doi.org/10.1007/s11356-014-3735-5>
- [6] Ahuja, P., Rami, E., Singh, A., Pathak, D. (2024): Chapter 8-Green synthesis of silver nanoparticles and their potential biological applications. In *Nanotechnol and In Silico Tools* (pp. 97-115). Elsevier. <https://doi.org/10.1016/B978-0-443-15457-7.00022-8>
- [7] Yang, J., Wang, Q., Wang, C., Yang, R., Ahmed, M., Kumaran, S., Velu, P., Li, B. (2020): *Pseudomonas aeruginosa* synthesized silver nanoparticles inhibit cell proliferation and induce ROS mediated apoptosis in thyroid cancer cell line (TPC1). *Artificial Cells Nanomedicine and Biotechnology* 48(1): 800-809. <https://doi.org/10.1080/21691401.2019.1687495>
- [8] Zaki, S., El Kady, M.F., Abd-El-Haleem, D. (2011): Biosynthesis and structural characterization of silver nanoparticles from bacterial isolates. *Materials Research Bulletin* 46(10): 1571-1576. <https://doi.org/10.1016/j.materresbull.2011.06.025>
- [9] Sosnowska, M.E., Jankiewicz, U., Kutwin, M., Chwalibog, A., Gałazka, A. (2018): Influence of salts and metal nanoparticles on the activity and thermal stability of a recombinant chitinase from *Stenotrophomonas maltophilia* N4. *Enzyme Microbial Technology* 116: 6-15. <https://doi.org/10.1016/j.enzmictec.2018.05.003>

- [10] Malhotra, A., Dolma, K., Kaur, N., Rathore, Y.S., Mayilraj, S., Choudhury, A.R. (2013): Biosynthesis of gold and silver nanoparticles using a novel marine strain of *Stenotrophomonas*. *Bioresource Technology* 142: 727-731. <https://doi.org/10.1016/j.biortech.2013.05.109>
- [11] Nayaka, S., Chakraborty, B., Airodgi, D., Bhat, M.P., Nagaraja, S.K., Swamy, P.S., R., M., Hiremath, H., Basavarajappa, S.D., Rego, M. (2020): In-vitro antimicrobial activity of biological synthesized silver nanoparticles using *Stenotrophomonas maltophilia* strain NS-24 from non-rhizosphere soil. *International Journal of Pharmacy and Pharmaceutical Sciences* 12: 73-79. <http://dx.doi.org/10.22159/ijpps.2020v12i5.37257>
- [12] Jang, E.Y., Son, Y.J., Park, S.Y., Yoo, J.Y., Cho, Y.N., Jeong, S.Y., Liu, S., Son, H.J. (2018): Improved biosynthesis of silver nanoparticles using keratinase from mages *Stenotrophomonas maltophilia* R13: reaction optimization, structural characterization, and biomedical activity. *Bioprocess and Biosystems Engineering* 41: 381-393. <https://doi.org/10.1007/s00449-017-1873-0>
- [13] Pernas-Pleite, C., Conejo-Martínez, A.M., Marín, I., Abad, J.P. (2022): Green extracellular synthesis of silver nanoparticles by *Pseudomonas allopuntida*, their growth and biofilm-formation inhibitory activities and synergic behavior with three classical antibiotics. *Molecules* 27(21): 7589. <https://doi.org/10.3390/molecules27217589>
- [14] Badoei-dalfard, A., Shaban, M., Karami, Z. (2019): Characterization, antimicrobial, and antioxidant activities of silver nanoparticles synthesized by uricase from *Alcaligenes faecalis* GH3. *Biocatalysis and Agricultural Biotechnology* 20: 101257. <https://doi.org/10.1016/j.bcab.2019.101257>
- [15] Rolim, W.R., Lamilla, C., Pieretti, J.C., Díaz, M., Tortella, G.R., Cristina Diez, M., Barrientos, L., Seabra, A.B., Rubilar, O. (2019): Comparison of antibacterial and antibiofilm activities of biologically synthesized silver nanoparticles against several bacterial strains of medical interest. *Energy Ecology and Environment* 4: 143-159. <https://doi.org/10.1007/s40974-019-00123-8>
- [16] Ramalingam, V., Rajaram, R., PremKumar, C., Santhanam, P., Dhinesh, P., Vinothkumar, S., Kaleshkumar, K. (2014): Biosynthesis of silver nanoparticles from deep sea bacterium *Pseudomonas aeruginosa* JQ989348 for antimicrobial, antibiofilm, and cytotoxic activity. *Journal of Basic Microbiology* 54(9): 928-936. <https://doi.org/10.1002/jobm.201300514>
- [17] Lavecachia, R., García-Martínez, J.B., Contreras-Roperio, J.E., Barajas-Solano, A.F., Zuurro, A. (2024): Antibacterial and Photocatalytic Applications of Silver Nanoparticles Synthesized from *Lactocaseibacillus rhamnosus*. *International Journal of Molecular Sciences* 25(21): 11809. <https://doi.org/10.3390/ijms252111809>
- [18] Alfryyan, N., Kordy, M.G., Abdel-Gabbar, M., Soliman, H.A., Shaban, M. (2022): Characterization of the biosynthesized intracellular and extracellular plasmonic silver nanoparticles using *Bacillus cereus* and their catalytic reduction of methylene blue. *Scientific Reports* 12(1): 12495. <https://doi.org/10.1038/s41598-022-16029->
- [19] AshaRani, P.V., Low Kah Mun, G., Hande, M.P., Valiyaveetil, S. (2009): Cytotoxicity and genotoxicity of silver nanoparticles in human cells. *ACS Nano* 3(2): 279-290. <https://doi.org/10.1021/nn800596w>
- [20] Takáč, P., Michalková, R., Čizmaríková, M., Bedlovičová, Z., Balážová, L., Takáčová, G. (2023): The role of silver nanoparticles in the diagnosis and treatment of cancer: Are there any perspectives for the future?. *Life* 13(2): 466. <https://doi.org/10.3390/life13020466>
- [21] Singh, P., Pandit, S., Balusamy, S.R., Madhusudan, M., Singh, H., Amsath Haseef, H.M., Mijakovic, I. (2025): Advanced nanomaterials for cancer therapy: gold, silver, and iron oxide nanoparticles in oncological applications. *Advanced Healthcare Materials* 14(4): 2403059. <https://doi.org/10.1002/adhm.202403059>
- [22] Kiliç, N.K., Kürkcü, G., Kumruoğlu, D., Dönmez, G. (2015): EPS production and bioremoval of heavy metals by mixed and pure bacterial cultures isolated from Ankara Stream. *Water Science and Technology* 72(9): 1488-1494. <https://doi.org/10.2166/wst.2015.365>
- [23] Keskin, N.O.S., Kılıç, N.K., Dönmez, G., Tekinay, T. (2016): Green synthesis of silver nanoparticles using cyanobacteria and evaluation of their photocatalytic and antimicrobial activity. *Journal of Nano Research* 40: 120-127. <https://doi.org/10.4028/www.scientific.net/JNanoR.40.120>
- [24] Behravan, M., Panahi, A.H., Naghizadeh, A., Ziaee, M., Mahdavi, R., Mirzapour, A. (2019): Facile green synthesis of silver nanoparticles using *Berberis vulgaris* leaf and root aqueous extract and its antibacterial activity. *International Journal of Biological Macromolecules* 124: 148-154. <https://doi.org/10.1016/j.ijbiomac.2018.11.101>
- [25] Tarannum, N., Gautam, Y.K. (2019): Facile green synthesis and applications of silver nanoparticles: a state-of-the-art review. *RSC Advances* 9(60): 34926-34948. <https://doi.org/10.1039/C9RA04164H>
- [26] Kılıç, N.K., Nielsen, J.L., Yüce, M., Dönmez, G. (2007): Characterization of a simple bacterial consortium for effective treatment of wastewaters with reactive dyes and Cr(VI). *Chemosphere* 67:826–831.
- [27] Kordzangeneh, H., Jookar Kashi, F. (2022): A new *Bacillus paralicheniformis* sp. Tmas-01 as bioreactor for synthesis of Ag/AgCl composite—different effects of biological and Rodamin B dye decolorization, anticancer, genotoxic activity. *Achieves of Microbiology* 204(12): 706. <https://doi.org/10.1007/s00203-022-03317-7>

- [28] Fernandes, M., González-Ballesteros, N., da Costa, A., Machado, R., Gomes, A.C., Rodríguez-Argüelles, M.C. (2023): Antimicrobial and anti-biofilm activity of silver nanoparticles biosynthesized with *Cystoseira* algae extracts. *Journal of Biological Inorganic Chemistry*. 28(4): 439-450. <https://doi.org/10.1007/s00775-023-01999-y>
- [29] Jia, S., Yu, J.M., Zhai, L., Yang, C., Yang, T., Li, H., Pan, Z., Zheng, B., Xiong, W.W. (2023): Surface modification of organic hybrid indium sulfide via silver ion decoration with enhanced photocatalytic activity for degrading organic dyes and antibiotics. *Journal of Solid State Chemistry* 324: 124100. <https://doi.org/10.1016/j.jssc.2023.124100>
- [30] Nguyen, N.P.U., Dang, N.T., Doan, L., Nguyen, T.T.H. (2023): Synthesis of silver nanoparticles: from conventional to 'modern' methods—a review. *Processes* 11(9): 2617. <https://doi.org/10.3390/pr11092617>
- [31] Castañeda-Aude, J.E., Morones-Ramírez, J.R., De Haro-Del Río, D.A., León-Buitimea, A., Barriga-Castro, E.D., Escárcega-González, C.E. (2023): Ultra-small silver nanoparticles: A sustainable green synthesis approach for antibacterial activity. *Antibiotics* 12(3): 574. <https://doi.org/10.3390/antibiotics12030574>
- [32] Voorhees, P.W. (1984); The theory of Ostwald ripening. *Journal of Statistical Physics*, 38(1): 231–252. <https://doi.org/10.1007/BF01017860>
- [33] Johnson, L., Gray, D.M., Niezabitowska, E., McDonald, T.O. (2021): Multi-stimuli-responsive aggregation of nanoparticles driven by the manipulation of colloidal stability. *Nanoscale* 13(17): 7879-7896. <https://doi.org/10.1039/d1nr01190a>
- [34] Polte, J. (2015); Fundamental growth principles of colloidal metal nanoparticles—a new perspective. *CrystEngComm* 17(36): 6809-6830. <https://doi.org/10.1039/c5ce01014d>
- [35] Xu, R.B., Yang, X., Wang, J., Zhao, H.T., Lu, W.H., Cui, J., Cheng, C-L., Zou, P., Huang, W-W., Hu, X.L. (2012): Chemical composition and antioxidant activities of three polysaccharide fractions from pinecones. *International Journal of Molecular Sciences* 13(11): 14262-14277. <https://doi.org/10.3390/ijms131114262>
- [36] Janik, S., Lubinski, T., Kaluza, M., Mäntele, W. (2024): Photonic blood glucose measurement with infrared quantum cascade lasers: A novel non-invasive and pain-free glucometer for diabetes patients. *PhotonicsViews* 21(1): 56-59. <https://doi.org/10.1002/phvs.202400001>
- [37] Yin, I.X., Zhang, J., Zhao, I.S., Mei, M.L., Li, Q., Chu, C.H. (2020): The antibacterial mechanism of silver nanoparticles and its application in dentistry. *International Journal of Nanomedicine* 15: 2555-2562. <https://doi.org/10.2147/IJN.S246764>
- [38] Ávila-Avilés, R.D., Argueta-Figueroa, L., García-Contreras, R., Vilchis-Nestor, A.R. (2024): Synthesis of biogenic silver and gold nanoparticles from *Anemopsis californica* extract with antibacterial and cytotoxic activities. *Materials Today Communications* 38: 108071. <https://doi.org/10.1016/j.mtcomm.2024.108071>
- [39] Jain, A.S., Pawar, P.S., Sarkar, A., Junnuthula, V., Dyawanapelly, S. (2021): Bionanofactories for green synthesis of silver nanoparticles: Toward antimicrobial applications. *International Journal of Molecular Sciences* 22(21): 11993. <https://doi.org/10.3390/ijms222111993>
- [40] Kamer, A.M.A., El Maghraby, G.M., Shafik, M.M., Al-Madboly, L.A. (2024): Silver nanoparticle with potential antimicrobial and antibiofilm efficiency against multiple drug resistant, extensive drug resistant *Pseudomonas aeruginosa* clinical isolates. *BMC Microbiology* 24(1): 277. <https://doi.org/10.1186/s12866-024-03397-z>
- [41] Ferreira, M.L., Behrmann, I.C.L., Daniel, M.A., Sosa, G.L., Owusu, E., Parkin, I.P., Candal, R., Allan, E., Vullo, D.L. (2024): Green synthesis and antibacterial-antibiofilm properties of biogenic silver nanoparticles. *Environmental Nanotechnology Monitoring & Management*: 22: 100991. <https://doi.org/10.1016/j.enmm.2024.100991>
- [42] Leng, D., Li, Y., Zhu, J., Liang, R., Zhang, C., Zhou, Y., Li, M., Wang, Y., Rong, D., Wu, D., Li, J. (2020): The antibiofilm activity and mechanism of nanosilver- and nanozinc-incorporated mesoporous calcium-silicate nanoparticles. *International Journal of Nanomedicine* 15: 3921-3936. <http://doi.org/10.2147/IJN.S244686>
- [43] Fayyadh, A.A., Jaduaa Alzubaidy, M.H. (2021): Green-synthesis of Ag₂O nanoparticles for antimicrobial assays. *Journal of the Mechanical Behavior of Materials* 30(1): 228-236. <http://doi.org/10.1515/jmbm-2021-0024>
- [44] Torabi, S., Mansoorkhani, M.J.K., Majedi, A., Motevalli, S. (2020): Synthesis, medical and photocatalyst applications of nano-Ag₂O. *Journal of Coordination Chemistry* 73(13): 1861-1880. <https://doi.org/10.1080/00958972.2020.1806252>
- [45] Huang, K., Li, C., Zheng, Y., Wang, L., Wang, W., Meng, X. (2022): Recent advances on silver-based photocatalysis: Photocorrosion inhibition, visible-light responsivity enhancement, and charges separation acceleration. *Separation and Purification Technology* 283: 120194. <https://doi.org/10.1016/j.seppur.2021.120194>
- [46] Wang, X., Li, S., Yu, H., Yu, J., Liu, S. (2011): Ag₂O as a new visible-light photocatalyst: self-stability and high photocatalytic activity. *Chemistry—A European Journal* 17(28): 7777-7780. <https://doi.org/10.1002/chem.201101032>
- [47] Mikhailova, E.O. (2020): Silver nanoparticles: Mechanism of action and probable bio-application. *Journal of Functional Biomaterials* 11(4): 84. <https://doi.org/10.3390/jfb11040084>
- [48] Mechouche, M.S., Merouane, F., Messaad, C.E.H., Golzadeh, N., Vasseghian, Y., Berkani, M. (2022): Biosynthesis, characterization, and evaluation of antibacterial and photocatalytic methylene blue dye degradation activities of silver nanoparticles from *Streptomyces tuius* strain. *Environmental Research* 204: 112360. <https://doi.org/10.1016/j.envres.2021.112360>

- [49] Reshma, Shaik, H., Manohar, K., Mukesh, B., Boddu, R.R., Ittikyala, S., Somaiah, P.V. (2023): Synthesis, Characterization, Biological Activity of PEG Capped Silver Nanoparticles and Photocatalytic Degradation of Methylene Blue Dye using LC-MS Method. *Asian Journal of Chemistry* 35(11): 2715-2725. <https://doi.org/10.14233/ajchem.2023.27005>
- [50] Debela, D.T., Muzazu, S.G., Heraro, K.D., Ndalama, M.T., Mesele, B.W., Haile, D.C., Kitui, S.K., Manyazewal, T. (2021): New approaches and procedures for cancer treatment: Current perspectives. *SAGE Open Medicine* 9: 20503121211034366. <https://doi.org/10.1177/20503121211034366>
- [51] Plackal Adimuriyil George, B., Kumar, N., Abrahamse, H., Ray, S.S. (2018): Apoptotic efficacy of multifaceted biosynthesized silver nanoparticles on human adenocarcinoma cells. *Scientific Reports* 8(1): 14368. <https://doi.org/10.1038/s41598-018-32480-5>
- [52] Krzyzanowski, D., Kruszewski, M., Grzelak, A. (2021): Differential action of silver nanoparticles on abcb1 (Mdr1) and abcc1 (mrp1) activity in mammalian cell lines. *Materials* 14(12): 3383. <https://doi.org/10.3390/ma14123383>
- [53] El-Naggar, N.E.A., Hussein, M.H., El-Sawah, A.A. (2017): Bio-fabrication of silver nanoparticles by phycocyanin, characterization, in vitro anticancer activity against breast cancer cell line and in vivo cytotoxicity. *Scientific Reports* 7(1): 10844. <https://doi.org/10.1038/s41598-022-06492-1>
- [54] Wang, B., Hu, S., Teng, Y., Chen, J., Wang, H., Xu, Y., Wang, K., Xu, J., Cheng, Y., Gao, X. (2024): Current advance of nanotechnology in diagnosis and treatment for malignant tumors. *Signal Transduction Targeted Therapy* 9(1): 200. <https://doi.org/10.1038/s41392-024-01889-y>
- [55] Oves, M., Khan, M.S., Zaidi, A., Ahmed, A.S., Ahmed, F., Ahmad, E., Sherwani, A., Qwais, M., Azam, A. (2013): Antibacterial and cytotoxic efficacy of extracellular silver nanoparticles biofabricated from chromium reducing novel OS4 strain of *Stenotrophomonas maltophilia*. *PloS one* 8(3): e59140. <https://doi.org/10.1371/journal.pone.0059140>
- [56] Viswanathan, M., Arumugam, S., Thangavel, B. (2016): In vitro anticancer activity of silver nanoparticles synthesized by *Escherichia coli* VM1 isolated from marine sediments of encore southeast coast of India. *Enzyme and Microbial Technology* 95: 146-154. <https://doi.org/10.1016/j.enzmictec.2016.09.008>
- [57] Xu, J.J., Zhang, W.C., Guo, Y.W., Chen, X.Y., Zhang, Y.N. (2022): Metal nanoparticles as a promising technology in targeted cancer treatment. *Drug Delivery* 29(1): 664-678. <https://doi.org/10.1080/10717544.2022.2039804>
- [58] Kanipandian, N., Ramesh, R. (2024): Biosynthesis and Characterization of Silver Nanoparticles Using *Cleistanthus collinus*; Investigation on Molecular Mechanism of Apoptosis Cell Death Against Lung Cancer Cells. *Asian Pacific Journal of Cancer Biology* 9(4): 527-535. <https://doi.org/10.31557/apjcb.2024.9.4.527-535>
- [59] Nadhe, S.B., Tawre, M.S., Agrawal, S., Chopade, B.A., Sarkar, D., Pardesi, K. (2020): Anticancer potential of AgNPs synthesized using *Acinetobacter* sp. and *Curcuma aromatica* against HeLa cell lines: A comparative study. *Journal of Trace Elements in Medicine and Biology* 62: 126630. <https://doi.org/10.1016/j.jtemb.2020.126630>