“Characterization, Cytotoxicity, and Genotoxicity Properties of Novel Biomediated Nanosized-Silver By Egyptian *Streptomyces Roseolus* For Safe Antimicrobial Applications”

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Abstract

For biosafety purposes, the genotoxicity of biosynthesized silver nanoparticles (Ag-NPs) was assessed using comet assay for the first time on Blood Lymphocytes, with zero tail migration and 100% head intensity indicating non-genotoxic effect. Ag-NPs have been projected as a new generation of antimicrobial agents because of its antimicrobial property. Ag-NPs were biomediated by Egyptian *Streptomyces roseolus* for the first time, that was molecularly identified using 16S rRNA sequencing under accession no. MT071505. Biosynthesized Ag-NPs were characterized using UV-Vis spectroscopy, XRD, TEM, FTIR, and DLS. FTIR confirmed the presence of different bioactive functional groups, such as, O-H, N-H, C-H, C-O-C, C-NH₂, and C=O acting as reducing-stabilizing agents for biosynthesized Ag-NPs. Biosynthesized Ag-NPs exhibited antimicrobial activity against some multi-drug resistant Gram-positive and Gram-negative pathogens. MBC of biosynthesized Ag-NPs against *Listeria monocytogenes* and *Klebsiella pneumonia* were 0.195 and 0.048 mg/mL, respectively, with tolerance level of 2 confirming its biocidal effect. SEM imaging of Ag-NPs-treated *L. monocytogenes* and *K. pneumonia* showed shrunk destroyed cells after 6h. Biosynthesized Ag-NPs exhibited IC₅₀ of <0.3 and 8.21 mg/mL, respectively, on normal Human Skin Fibroblast, and Blood Lymphocytes. IC₅₀ values were significantly higher than its MBC values, with no harmful cytotoxic-effect, thus can be safely applied at its biocidal concentration. An ecofriendly biomediated synthesis of Ag-NPs was described with easy scale-up, non-toxic by-products, so, it can be recommended as powerful-safe antimicrobial agent.

Highlights

- Biosynthesized Ag-NPs has no genotoxicity on peripheral Blood Lymphocytes using comet assay.
- Ag-NPs were biomediated by new Egyptian isolate *Streptomyces roseolus* for the first time.
- Biosynthesized Ag-NPs were characterized using UV-Vis, XRD, TEM, FTIR, and DLS analysis.
- FTIR confirmed the presence of different bioactive functional groups, acting as reducing/ stabilizing agents for the Ag-NPs.
- Biosynthesized Ag-NPs exhibited antimicrobial activity against some multi-drug resistant Gram-positive and Gram-negative pathogens.
- No cytotoxic effect of biosynthesized Ag-NPs, with significantly high IC₅₀ and no harmful effect on normal Blood Lymphocytes and Human Skin cell lines.

Introduction

Due to the antigenic changes of multi-drug resistant bacteria, they are not effectively managed with current treatments, making it a serious global problem for public health (Wypij et al. 2018). Accordingly, there is a strong need to develop new bactericides. In this respect, metallic nanoparticles (NPs) has gained a great significance in the modern era of nanomaterials because of their unique intrinsic properties based on their morphology, size, and distribution (Mourdikoudis et al. 2018). These properties are excellent physicochemical, optical, electrical conductivity, catalytic, and mechanical stability (Srikar et al. 2016; Crisan et al. 2021). Among metallic NPs, Ag-NPs have found tremendous applications in the field of catalysis, biosensing, drug delivery, and medicine, etc. Recently, there has been an increasing interest in Ag-NPs because of its antimicrobial property, and even being projected as a new generation of antimicrobial agents (Martínez et al. 2021). Therefore, Ag-NPs have been exploited for various applications, such as antimicrobial agents, in household and healthcare-related products, wound dressings, medical device coatings, cosmetics (Zhang et al. 2016; Wypij et al. 2018; Vanlalveni et al. 2021).

Although, physicochemical synthesis generates stable monodispersed NPs with various shapes, these methods aren't recommended, because they involve the use of chemical reagents, require costly reaction setup, and generate toxic end-products causing environmental pollution (Srikar et al. 2016; Yaqoob et al. 2020). These drawbacks, make it very important to search for eco-friendly, safe, and cost-effective approaches for the synthesis of NPs; alternative to chemical/physical synthesis. Interestingly, microorganisms are considered as nano-factories that play vital role in the bioremediation of toxic metals through the reduction of metal ions (Anandaradje et al. 2020). In this respect, the Actinomycetes members of the bacterial order Actinomycetales are found worldwide in soil, where *Streptomyces*, the largest genus of Actinobacteria are considered efficient candidates for the biosynthesis of metallic NPs with very good stability (Wypij et al. 2018; Garg et al. 2020).

Furthermore, microorganisms or their secondary metabolites contains different biomolecules, which act as reducing and/or stabilizing agents for Ag-NPs and represents safe approach than the physicochemical methods (Anandaradje et al. 2020). These natural reducing agents contain many functional groups which can increase the reduction capability and improve Ag-NPs stability (Zhang et al. 2016). Herein, the main objective of this study was to achieve a green approach for the biosynthesis of Ag-NPs using new *Streptomyces* strain isolated from the Egyptian soil for the first time, and molecularly identified using 16S rRNA gene sequencing. Characterization of the produced Ag-NPs was investigated using UV-Vis, TEM, DLS, XRD, and FTIR analysis. The antimicrobial activity of the biosynthesized Ag-NPs was tested against different Gram-positive, Gram-negative and yeast pathogens. The effect of the biosynthesized Ag-NPs on *Listeria monocytogenes* and *Klebsiella pneumonia* was assessed using SEM. For biosafety applications, the cytotoxicity effect of biosynthesized Ag-NPs has been tested on HepG-2, peripheral Blood Lymphocytes, and on normal Human Skin Fibroblast cell lines. And for the first time the genotoxicity of the biosynthesized Ag-NPs was assessed using comet assay on peripheral Blood Lymphocytes.

Material And Methods

Materials

AgNO₃ as a precursor for Ag-NPs synthesis was obtained from Sigma/Aldrich, USA. Peptone, Yeast extract, Malt extract, Glucose, Starch, and Dimethyl Sulphoxide (DMSO) were purchased from Lobal Chemie, India. All materials used for cell culture experiments were obtained from Cambrex BioScience (Copenhagen, Denmark). All other chemicals and reagents were bought locally and were of analytically reagent grade from Algomhoryia Company for Chemicals, Cairo, Egypt.

Culture media and Microorganisms

Glucose Yeast Malt medium was used for actinomycetes isolation having the following composition (g/L); malt extract 10, yeast extract 3, glucose 3, agar 20, and the pH was adjusted to 7.3±0.1 at 25°C. Starch nitrate medium was used as a synthetic medium for biosynthesis of Ag-NPs (Abd-Elnaby et al. 2016) with the following composition (g/L); starch 20, K₂HPO₄ 0.5, KNO₃ 1, MgSO₄·7H₂O 0.5, FeSO₄ 0.01, KCl 0.5, and pH was adjusted to 7.2±0.2 at 25°C. Nutrient agar medium was used for growth and maintenance of pathogens with the following composition (g/L); beef extract 3, peptone 5, NaCl 5, agar 20, and pH 7.

Gram-positive bacteria (*Bacillus cereus* ATCC 14579, *Bacillus subtilis* ATCC 6633, *Listeria monocytogenes*, *Staphylococcus aureus* ATCC 6538), gram-negative strains (*Escherichia coli* O157:H7, *Klebsiella pneumonia*, *Aeromonas hydrophilia*), and Yeast (*Candida albicans*), were obtained from Dar Al-Fouad Hospital at 6th October city, Cairo, and El-Mabarra Educational Hospital, Alexandria, Egypt.

Isolation of actinomycetes

Soil samples were collected from Menuf, Minufyia governorate, Egypt in sterile plastic bags. 10 g of collected sample was suspended in 90 mL of saline solution (0.9% NaCl, w/v), serially diluted, and one mL aliquot of each dilution was plated on sterile agar plates containing Glucose Yeast Malt Agar medium and were incubated for 5 days at 30°C. Colonies which were morphologically identified as actinomycetes were selected. Pure developed single colonies were obtained by conventional streak plate technique, kept at 4°C on slants. All cultures were routinely stored in glycerol solution (25% v/v) at -80°C.

Biosynthesis of Ag-NPs

Cell free extract of the isolated actinomycete was grown in 250 mL-Erlenmeyer flask containing starch nitrate broth medium, incubated in a rotary shaker at 170 rpm and 30°C for three days. After the incubation, culture was centrifuged at 6000 rpm for 20 min, and the collected supernatant was used for the biosynthesis of Ag-NPs. 50 mL of AgNO₃ solution (1 mM) were mixed with 50 mL of the actinomycete supernatant (Abd-Elnaby et al. 2016). The mixture was incubated at 30°C and 170 rpm agitation in a rotary shaker, for 24 h in dark. To study the temperature effect on the biosynthesis, the reaction mixtures were incubated at different temperature degrees (30, 45, and 60°C) for 24 h at 150 rpm in rotatory shaker. Control experiments were carried out by mixing AgNO₃ solution and un-inoculated media or adding the actinomycete supernatant with double distilled H₂O without AgNO₃. The reduction of Ag⁺ ions was checked by color change of the reaction mixture from yellow to brown.

Characterization of biosynthesized Ag-NPs

UV-Visible spectroscopy (UV-Vis)

Formation of Ag-NPs during the biosynthesis was monitored based on surface-plasmon-resonance using a UV-Vis spectrophotometer (Shimadzu T80 spectrophotometer, China). One mL aliquot of biosynthesized Ag-NPs colloidal solution was scanned at wavelength ranging from 300 to 700 nm.

X-ray diffraction (XRD)

Crystalline natures of the dried biosynthesized Ag-NPs powder were investigated by X-ray diffraction (Bruker D2 Phaser diffractometer 2nd Gen.), operating at (50 kV and 0.5 mA), with a Cu anode radiation (1.54060 Å) in the angular range of 10°-70° using continuous scanning 2 θ mode. The size of the Ag-NPs formed in the bio-reduction process was determined using Scherrer's formula (Sumadevi et al. 2021).

$$D = K \lambda / \beta \cos \theta$$

where **K** is the Scherrer constant (shape factor), **λ** is the X-ray wavelength (1.5418 Å), **β** is the width of the XRD peak at half-height, **θ** is the Bragg angle, and **D** is the grain size.

Transmission electron microscope (TEM)

The size and morphology of the biosynthesized Ag-NPs was studied by TEM (FETEM, JSM-2100F, JEOL Inc.) at Petroleum Research Institute, Cairo, Egypt. An aliquot of Ag-NPs suspension was transferred onto carbon-coated copper grid, and allowed to dry. The grid was then scanned using TEM (Phillips EM 208S) operated at 100 kV voltage.

Dynamic light scattering (DLS)

Biosynthesized Ag-NPs was analyzed using Nano-Zeta Sizer (Nano ZS, ZEN 3600, Malvern Nano, UK) to determine the distribution of particles size. DLS measures the light scattered from a laser when passes through sample that mostly

depends on Rayleigh scattering from the suspended NPs and the fluctuations of the scattered light are detected at a known scattering angle θ by a fast photon detector (Fissan et al. 2014).

Fourier transform infrared (FTIR)

The biosynthesized Ag-NPs were dried then diluted (1:100) by potassium bromide. The FTIR spectrum of the sample was recorded on a FTIR spectrometer 8000 series, with KBr in the wavenumber region of 4,000-400 cm^{-1} at a resolution of 4 cm^{-1} . To identify the functional groups found in the tested sample, the spectral data recorded were compared with the reference data chart.

Molecular identification and phylogenetic analysis

The selected actinomycetes isolate was identified using 16S rRNA gene partial sequencing method (Kim et al. 2011). Genomic DNA of the selected actinomycetes isolate was extracted using genomic DNA extraction Kit (Intron, Biotechnology, Korea). PCR amplification of 16S ribosomal DNA (16S rDNA) was performed with a set of universal primers; **27F-Forward primer 5'AGA GTT TGA TCC TGG CTC AG 3'** (20 mer) and **1492R-Reverse primer 5'CTA CGG CTA CCT TGT TAC GA 3'** (20 mer). The PCR amplification was performed using programmed thermal cycler (Thermo Fisher Scientific, USA). The produced amplicons of the selected isolate (PCR product) was tested for its quality by electrophoresis in Bio-Rad submarine (8x12 cm) using agarose gel (1%) and compared with 1kb DNA ladder (Intron Biotechnology, Korea). DNA banding patterns of 16S gene amplicons were visualized using a UV-transilluminator (Thermo Fisher scientific, USA) under UV light. PCR products were purified by using gene JET™ genomic DNA purification kit (Intron Biotechnology, Korea) according to the manufacturer's instructions. Then sequenced using forward and reverse primers with ABI 3730xl DNA sequencer. Nucleotide bases obtained after sequencing were identified and compared with similar sequences retrieved from the GenBank database within the National Center for Biotechnology information (NCBI) (<http://www.ncbi.nlm.nih.gov/GenBank/index.html>) using nucleotide Basic Local Alignment Search Tool (BLAST) Gene Sequences in the database website (<http://www.ncbi.nlm.nih.gov/BLAST/>). The nucleotide sequences of the 16S rRNA genes was deposited under accession number MT071505, and the phylogenetic analysis of sequences was created using Alignment Search Tools (BLAST).

Qualitative and quantitative assessment of antimicrobial activity

Bacterial strains and growth conditions

The antimicrobial activity of the biosynthesized Ag-NPs was tested against different Gram-positive bacteria (*L. monocytogenes*, *S. aureus*, *B. subtilis*, *B. cereus*), Gram-negative bacteria (*E. coli* O157:H7, *K. pneumonia*, *A. hydrophilia*), and yeast (*C. albicans*). All bacterial strains were grown on nutrient broth, except *L. monocytogenes* was grown on brain heart infusion (Oxoid, Ltd, England) and the yeast was grown on Wickerham medium. Stock inoculum suspensions of the pathogenic strains were freshly prepared by picking colonies from 24 h cultures grown on at 37°C and suspended in sterile saline solution (0.9% NaCl, w/v). The optical density of pathogens was adjusted to achieve turbidity equivalent to 0.5 McFarland standard, approximately (1×10^8 CFU/mL) for bacteria, and (1×10^6) for *C. albicans* (Andrews 2001).

Disc diffusion method

Disc diffusion method is the common method used as a preliminary screening test prior to quantitative minimal inhibitory concentration (MIC) determination. Microbial inocula were spread on plates containing appropriate medium using sterile cotton swab, and plates were allowed to dry for 15 min at room temperature. Sterile filter paper discs (Hi media) were saturated by 40 μL of two-fold serially diluted concentrations of biosynthesized Ag-NPs in the range (0.39-

25 mg/mL). Discs saturated with the actinomycete supernatant and AgNO₃ were used as a negative and positive control, respectively. The plates were incubated at 4°C for 1h to allow diffusion of Ag-NPs into agar medium, and were incubated at 37°C for 24h (Wikler 2006). After incubation, inhibition zones diameter was measured in mm, and MIC was determined as the lowest concentration of biosynthesized Ag-NPs that produced an inhibition zone after 24h of incubation.

Agar dilution method

Brain Heart Infusion (BHI) and nutrient agar (NA) media were used for the growth of *L. monocytogenes* and *K. pneumonia*, respectively. Two-fold serial dilutions of biosynthesized Ag-NPs were prepared in molten BHI agar and NA medium, cooled down to 45°C with desired final concentrations. Using pour-plate method (Klančnik et al. 2010), 100 µL of *L. monocytogenes* or *K. pneumonia* bacterial suspensions (0.5 Mcfarland~1:2×10⁸ CFU/mL) were inoculated into sterilized agar petri-plates containing appropriate medium, and incubated at 37°C for 24-48 h. An inoculated agar plate without Ag-NPs was served as a positive control and another one without inoculum as a negative control.

Broth macro-dilution method, MIC, and MBC determination

Broth macro-dilution was done according to Andrews (2001) method. The biosynthesized Ag-NPs were added to 10 mL of BHI broth medium and nutrient broth to give final concentrations with respect to the results obtained by the agar dilution method. 100 µL from the diluted bacterial culture of *L. monocytogenes* or *K. pneumonia* (0.5~Mcfarland 1:2×10⁸ CFU/mL) were inoculated in growth media containing the desired concentration of Ag-NPs, well shaken, and incubated at 37°C for 24h. Bacterial growth was followed by plating 1 mL from the incubated culture tubes and control on suitable media after serial dilutions. After 24h of incubation, plates were compared with positive control and the bacterial colony number was calculated as (CFU/mL). The MIC is the lowest concentration of biosynthesized Ag-NPs resulting in significant reduction (99%) of pathogen viability after 24h of incubation. The concentration where 100% of microbial growth was inhibited as compared to the negative control (media only) was designed as MBC value. The tolerance level of pathogen towards the biosynthesized Ag-NPs was calculated using the following formula (May et al. 1998):

$$\text{Level of Tolerance} = \text{MBC/MIC}$$

Scanning electron microscope (SEM) imaging

The effect of biosynthesized Ag-NPs on pathogens was studied using SEM, micrographs were captured in a Jeol JSM-5300 SEM operated between 15 and 20 KeV at Faculty of Science, Alexandria University, Alexandria, Egypt. Samples were prepared according to Tamboli and Lee (2013) method with some modifications as follows; 10 mL of fresh bacterial culture of *L. monocytogenes* and *K. pneumonia* grown in Luria-Bertani medium for 18h were treated with biosynthesized Ag-NPs at the detected MIC, then incubated for 3 and 6h. Treated bacterial cells were collected by centrifuging at 6000 rpm for 10 min. Culture supernatants were discarded and the treated cells were washed by phosphate saline buffer (pH 7.4) to remove excess of Ag-NPs precipitated on the cells, then centrifuged to collect washed cells. Samples were fixed by immersing immediately in equal volume of a fixative solution (1% glutaraldehyde, 4% paraformaldehyde in 0.1M sodium-cacodylate) at 4°C for 24 h. Specimens were then post fixed in 2% OsO₄ in the phosphate saline buffer (pH 7.4) at 4°C for 2 h. Samples were washed in the buffer and dehydrated at 4°C through graded series of ethanol, and dried by N₂ gas. Finally, the samples were mounted using carbon paste on AL-stub using double-sided conductive tapes, and coated with gold up to a thickness of 400 Å in a sputter-coating unit (JFC-1100 E) provided at the SEM lab.

Cytotoxic assessment of biosynthesized Ag-NPs

Cell viability and cytotoxicity of biosynthesized Ag-NPs was tested on normal Human Skin Fibroblast (HSF) cell-lines using sulforhodamine B protein (SRB) assay, and on Peripheral Blood Lymphocytes normal cells, as well as Human Hepatocellular Carcinoma (HepG-2) cell-lines using MTT assay. Normal HSF cell-lines were obtained from Nawah Scientific Inc., (Mokatam, Cairo, Egypt). Cells were maintained in Dulbecco's Minimum Essential Medium (DMEM) supplemented with 100 mg/mL of streptomycin, 100 U/mL of penicillin and 10% of heat-inactivated fetal bovine serum in humidified CO₂ (5% v/v) at 37°C. Aliquots of 100 µL cell suspension (5x10³ cells) were distributed in 96-well plates and incubated for 24h. Cells were treated with aliquot of 100 µL media containing Ag-NPs (0-300 µg/mL) concentrations. After 72h of exposure, cells were fixed using 150 µL of TCA (10%), and incubated for 1h at 4°C. The TCA solution was removed, and cells were washed 5-times using double distilled water. Aliquots of 70 µL SRB solution (0.4% w/v) were added and incubated at 25°C in darkness for 10 min. Plates were washed 3 times with acetic acid (1% v/v) and allowed to air-dry. To dissolve the protein-bound SRB stain, 150 µL of TRIS (10 mM) was added, and the absorbance was measured at 540 nm using a BMGLABTECH®-FLUO star Omega microplate reader, Ortenberg, Germany (Skehan et al. 1990).

Hep-G2 cell-lines (ATCC, USA) were used to evaluate the cytotoxic effect of the biosynthesized Ag-NPs using 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were routinely cultured in DMEM, which is supplemented with 10% FBS, 2 mM L-glutamine, 100 U/mL streptomycin-sulphate, 250 ng/mL amphotericin B, and 100 U/mL sodium penicillin-G. All cells were maintained in humidified air containing 5% CO₂ at 37°C. For sub-culturing, the monolayer cells were harvested after trypsin/EDTA treatment at 37°C. Peripheral Blood Lymphocytes were isolated from fresh blood samples according to Kizhakeyil et al. (2019). Lymphocyte cell suspension was centrifuged at 2000 rpm for 10 min, washed twice then suspended in incomplete RPMI-1640. Lymphocyte cell viability was checked using Trypan blue stain (Sigma) and was above 90%. The cells (2.5x10⁵ cells/mL) were cultured in RPMI-1640 supplemented with 10% FBS as triplicates of 200 µL/well into flat-bottom 96-well tissue culture plates (Griener), and incubated with serial concentrations of biosynthesized Ag-NPs (48-500 µg/mL concentration) for 48h in a humidified 5% CO₂ atmosphere. HepG-2 cells (0.5x10⁵ cells/well), in serum-free medium, were plated in a flat bottom 96-well microplate, and treated with 20 µL of different Ag-NPs concentrations in the range (62.5-500 µg/mL) for 24h at 37°C, in a humidified 5% CO₂. After incubation, 40 µL MTT solution/well were added after the removal of media and incubated for an additional 4h. MTT crystals were solubilized by the addition of 180 µL of DMSO/well, then plates were shaken at room temperature, followed by photometric determination of the absorbance at 570 nm using microplate ELISA reader (Sunrise™, Tecan Group Ltd. Männedorf/Switzerland). Each concentration was carried out in triplicate, the average and standard deviation were calculated. Data were expressed as the percentage (%) of relative viability compared with untreated cells, with cytotoxicity indicated by <100% relative viability. The extent of MTT reduction was quantified, where the number of viable cells was directly proportional to the intensity of formazan dark-blue color (Hansen et al. 1989). IC₅₀ was calculated from dose response curve and the percentage (%) of relative viability was calculated using the following equation:

$$[\text{Absorbance of treated cells} / \text{Absorbance of control cells}] \times 100$$

***In vitro* genotoxicity alkaline comet assay**

The comet assay was assessed according to Singh et al. (1988) with some modifications. Treated cells were incubated with different concentrations of biosynthesized Ag-NPs for 12h, under proper sterilized anaerobic conditions. Cells were washed by phosphate saline buffer (pH 7.4), resuspended in 0.75-1% low dissolving agarose (Bio-Rad) and were spread onto slides covered by 1% typical agarose. Slides were left to solidify at 40°C for 5 min, then were placed in lysis buffer (2.5M NaCl, 10 mM Tris, 100 mM EDTA, 1% TritonX100, 10% DMSO, pH 10) overnight at 40°C. Slides were placed in unwinding buffer (300 mM NaOH, 1 mM EDTA, pH 13) for 40 min at 40°C. Electrophoresis was carried out for 20 min

at 25V (1.0 V/cm), and 300 mA. Finally, the slides were neutralized using 0.4M Tris buffer (pH 7.5), and were stained with ethidium bromide (20 µg/mL), then examined using a fluorescence light microscope (Leica DMI8 S-platform, Germany), 100×objective lens. The tail intensity %, head intensity, and tail moment were assessed using Comet examine IV software.

Statistical analysis

All experiments were conducted in triplicates, the mean and all standard deviations were calculated using Excel software (Microsoft office, 2010). Data were expressed in their mean values ± SD (standard deviation).

Results

Biosynthesis of Ag-NPs

In this study, Ag-NPs were successfully synthesized extracellularly using the culture supernatant of new *Streptomyces* strain isolated from the Egyptian soil (Fig. 1). Interestingly, the culture supernatant incubated with 1 mM AgNO₃ mediated the biosynthesis of Ag-NPs within 6 h of incubation. The color of the reaction mixture was changed from yellow to dark reddish-brown, which indicates the formation of Ag-NPs (Fig. 1), whereas, no color change was observed in either the culture supernatant without AgNO₃ or un-inoculated media with AgNO₃ as control. After 24h, the stability of color indicated that AgNO₃ was completely reduced, and the reaction has come to an end.

Molecular identification and phylogenic tree of selected isolate

PCR amplification of the DNA samples from the selected *Streptomyces* isolate #140 generated a PCR product (Fig. 2) of expected size (1500 bp). The analysis of 16S rRNA gene of the *Streptomyces* isolate #140 was sequenced with 27F and 1492R primers at the forward and reverse directions which was compared with available 16S ribosomal sequences in NCBI GenBank database (<http://www.ncbi.nlm.nih.gov/GenBank/index.html>) using BLAST. The NCBI database showed 96.25% of similarity with *Streptomyces roseolus*, under accession no. MT071505. Gene bank nucleotide database using the blast-n algorithm revealed significant matches with hi max score of 2026, zero e-value, and 96.25% nucleotide identity for forward 16s rRNA gene sequence of *Streptomyces* isolate #140 and that of *Streptomyces roseolus* strain (NBRC 12816). Also, the reverse sequence shows hi max score of 2034 and zero e-value with 96.91% identity with *S. roseolus* strain (NBRC 12816). The phylogenetic tree (Fig. 2) shows high genetic relationship between the Egyptian *Streptomyces* isolate #140 and *S. roseolus* strain (NBRC 12816) with 0.003 genetic relationship.

Characterization of biosynthesized Ag-NPs

A strong and broad surface-plasmon-resonance (SPR) band maximum was observed at 470 nm, characteristic for Ag-NPs (Fig. 1). The color intensity was increased with increasing the temperature (Fig. 1), and consequently, the intensity of SPR peaks were increased. The XRD pattern of the biosynthesized Ag-NPs by *S. roseolus* (Fig. 1) showed characteristic XRD diffraction peaks centered at 37.874, 43.734, 64.277 induced by the following crystalline planes of Ag, 111, 200, and 220, respectively, as indexed planes of cubic face-centered silver. Other peaks were attributed to the presence of organic substances in culture supernatant. Peaks intensity reflects the high degree of Ag-NPs crystallinity. According to Scherrer's equation, the average size of Ag-NPs was found to be 10.8 nm. TEM micrograph showed the formation of well-dispersed spherical Ag-NPs biosynthesized by *S. roseolus* (Fig. 3). The size of Ag-NPs determined by TEM was 5-22 nm. The DLS pattern demonstrated that Ag-NPs biosynthesized by *S. roseolus* had a Z average diameter of 56.33 nm, and its poly-dispersity index (PDI) was 0.398. Fig. (3) shows a bimodal scattered intensity distribution with a major maximum at about 56 nm. The FTIR analysis (Fig. 4) verified the presence of different functional groups on the

surface of biosynthesized Ag-NPs. Results shows specific strong absorption bands at 3420.14, 2952, 1614, and 1430 cm^{-1} . Different absorbance peaks like 1370, 1154, and 1062 cm^{-1} were also detected.

Antimicrobial activity of Ag-NPs

The biosynthesized Ag-NPs exhibited an excellent dose-dependent antimicrobial activity against different Gram-negative, Gram-positive bacteria, and yeast (*C. albicans*) (Fig. 5 and Table 1). The highest antimicrobial activity was against Gram-negative bacteria. Among all pathogenic strains, the biosynthesized Ag-NPs exhibited a higher antibacterial activity against *E. coli* O157:H7 with maximum inhibition zone diameter of 24 mm (Table 1). The lowest activity was against *C. albicans* and *B. subtilis* with inhibition zone of 18.66 and 19.66 mm, respectively.

Quantitative determination of MIC, MBC, and tolerance level

Table (2) shows that the MIC values obtained by agar dilution and broth macro-dilution methods were 195 and 97 $\mu\text{g/mL}$, respectively, for *L. monocytogenes*. Furthermore, the MBC values obtained by these two methods were very applicable at concentration of 195 $\mu\text{g/mL}$ for the same strain. However, a lower MIC values of biosynthesized Ag-NPs was sufficient for the inhibition of Gram-negative *K. pneumonia* at 48 and 24 $\mu\text{g/mL}$, and MBC values were 97 and 48 $\mu\text{g/mL}$, using agar dilution and broth macro-dilution, respectively. Results also indicated that the Gram-negative *K. pneumonia* was more sensitive to Ag-NPs than the Gram-positive *L. monocytogenes*. Results in Table (2) shows that broth macro-dilution method is more sensitive than other screening agar methods, thus more suitable for rapid quantitative determination of the antimicrobial activity of Ag-NPs. Using broth macro-dilution method, the antimicrobial activity expressed as MIC was at the same or at lower concentrations as compared to the agar dilution methods.

The tolerance level of pathogens towards Ag-NPs was calculated from their respective MIC and MBC values (Table 2). The tolerance level was 2 using broth macro-dilution method, for *L. monocytogenes*, and *K. pneumonia*. In the current study, both MIC and MBC determined using the two dilution methods did not observe any visible growth when plated on solid media after 24 h of incubation, and the MBC was equal to or within 1 or 2 double its MIC.

SEM imaging

Cells treated with their MIC of biosynthesized Ag-NPs after 3 and 6h of incubation showed notable morphological alterations as compared to the control with no treatment (Fig. 6). After 3h of exposure to MIC concentrations, the treated *L. monocytogenes* and *K. pneumonia* cells were tiny, shrunk, diminutive, and dehydrated, with many pits in its surface. Some cells showed large leakage, others appeared misshapen and fragmented. After 6h of exposure to Ag-NPs, the microbial cells were disrupted, fully lysed, and the interior dehydrated cellular components and debris was the only observable matrix.

Cytotoxicity assessment

The cytotoxicity of tested samples was measured against Hep-G2 malignant cell-lines and Peripheral Blood Lymphocytes (normal cells) using the MTT assay, as well as against Human Skin Fibroblast (HSF) normal cell lines using the SRB assay. Analysis of cytotoxicity by MTT assay assured the non-cytotoxic impact of biosynthesized Ag-NPs on normal tested cells (Fig. 7 and Table 3). Results revealed that the biosynthesized Ag-NPs has no cytotoxic effect on different cell lines in the tested range (48-500 $\mu\text{g/mL}$). The cytotoxic effect of biosynthesized Ag-NPs showed IC_{50} of 1147 $\mu\text{g/mL}$ for HepG-2, 8210 $\mu\text{g/mL}$ for normal Peripheral Blood Lymphocytes, and >300 $\mu\text{g/mL}$ for HSF normal cell lines. The biosynthesized Ag-NPs has no harmful effect on the cell viability of normal Blood Lymphocytes which was almost the same (>90% viability) for all tested concentrations. Overall, the biosynthesized Ag-NPs (Fig. 7 and Table 3)

had significantly higher IC₅₀ than its MBC values (97-195 µg/mL) against the two studied multidrug-resistant pathogens (*K. pneumonia* and *L. monocytogenes*).

Genotoxicity assessment

Comet assay was performed on treated peripheral Blood Lymphocytes to evaluate the genotoxic potential of biosynthesized Ag-NPs. Results showed no fragmentation of DNA in Blood Lymphocytes cells and no tail formation was observed for all tested concentrations using comet electrophoresis (Fig. 8). This is the first report showing the genotoxicity assessment of biosynthesized Ag-NPs on peripheral Blood Lymphocytes. No genotoxic was recorded in all tested samples and data obtained (Fig. 8) showed that tail migration is zero and head intensity is 100%, which indicates that there was no genotoxic effect of the biosynthesized Ag-NPs on treated cells.

Discussion

Biosynthesis of Ag-NPs, molecular identification and phylogenic tree of the selected isolate

Biological synthesis of Ag-NPs has received great attention owing to their eco-friendly and cost-effective process. The surface of Ag-NPs are coated with different biomolecules from culture supernatant making them biocompatible as compared to those prepared by chemical methods. Therefore, biological synthesis offers interesting applications in biomedicine and other related fields (Sharma et al. 2019). In this study, Ag-NPs were successfully synthesized extracellularly using the culture supernatant of new *Streptomyces* strain isolated from the Egyptian soil within 6 h only. The new selected isolate was molecularly identified using 16S rRNA gene, where the sequencing of the highly conserved 16S rRNA gene represents one of the most commonly used techniques for identifying microorganisms. It is a powerful, simple, and fast method for determining evolutionary and phylogenetic relationships among microorganisms (Hu et al. 2018). The NCBI database and phylogenetic tree showed 96.25% similarity with *Streptomyces roseolus* strain (NBRC 12816), with high genetic relationship confirming its identity as *Streptomyces roseolus*.

UV-Vis spectroscopy, TEM, DLS, XRD, and FTIR Characterization

The strong and broad surface-plasmon-resonance (SPR) band maximum that was observed at 470 nm, is characteristic for Ag-NPs. The intensity of SPR peaks detected by UV-Vis spectroscopy was increased which is probably due to the increase in Ag-NPs concentration (Ibrahim 2015). Similarly, Alsamhary (2020) reported that Ag-NPs were biosynthesized using *B. subtilis* with strong broad peak between 400-470 nm indicating the formation Ag-NPs. DLS measures the hydrodynamic diameter of NPs, which is higher than its actual diameter obtained from TEM images. By relating the number % with its PDI it is suggested that the produced Ag-NPs were highly dispersive. Likewise, El-Bendary et al. (2021) synthesized Ag-NPs using *B. subtilis* isolate NRC1 (MK333152.1) which showed a single peak with an average hydrodynamic diameter size of 20 nm and PDI of 0.

The XRD pattern of the biosynthesized Ag-NPs by *S. roseolus* showed characteristic XRD diffraction peaks as indexed planes of cubic face-centered silver. Peaks intensity reflects the high degree of Ag-NPs crystallinity. Other peaks were attributed to the presence of organic substances in culture supernatant. The XRD spectrum was compared with the standard sample published by the Joint Committee on Powder Diffraction Standards (file no. 04-0783), which confirmed the crystalline nature of produced Ag-NPs. Likewise, Bakhtiari-Sardari et al. (2020) synthesized Ag-NPs using *Streptomyces* sp. OSIP1 with diffraction peaks typical to metal face-centered cubic silver at 38.2 (111), 44.4 (200), 64.6 (220), and 77.5 (311) corresponding to the crystalline planes of Ag. The FTIR analysis verified the presence of different functional groups on the surface of biosynthesized Ag-NPs due to the presence of different biomolecules in the *Streptomyces* supernatant that act as reducing, and stabilizing agent for the produced Ag-NPs (Zhang et al. 2016). Specific strong absorption bands at 3420.14 cm⁻¹ (N-H stretch) was characteristic to the amine group (Mourdikoudis et

al. 2018). The strong band at 2952 cm^{-1} was assigned to aldehydic C-H stretching (Sharma et al. 2019). Bands at 1614 cm^{-1} and 1430 cm^{-1} were assigned to absorption peaks of (C=C) alkyl and (-C-H) alkanes stretch, respectively (Kannan et al. 2013; Mourdikoudis et al. 2018). Different absorbance peaks at 1370 , 1154 , and 1062 cm^{-1} were related to CH_2 of alkanes, C-O of carboxylic acid, and (-C-N) stretching, respectively (Ghaseminezhad et al. 2012; Ibrahim 2015). The amide and aromatic group residues of proteins have a stronger ability to bind metals and form a surface layer on metallic Ag-NPs confirming that proteins act as capping agent for stabilizing the biosynthesized Ag-NPs (Malarkodi et al. 2013).

Antimicrobial activity of Ag-NPs, MIC, and MBC determination

Ag-NPs have a significant advantage as compared to conventional chemical antimicrobial agents towards multidrug resistance problem. Chemical antimicrobial agents are limited to use, because various microorganisms have established several resistance properties over generations. Consequently, the development of Ag-NPs can be an alternative approach to overcome the multidrug resistant microorganisms, and this because bacteria are less probable to develop resistance to metallic NPs as compared to conventional antibiotics (Loo et al. 2018).

The highest antimicrobial activity was against Gram-negative which is probably due to the cell wall structure (Erjaee et al. 2017). Similarly, Huq and Akter (2021) reported that the extracellularly biosynthesized Ag-NPs by *Paenarthrobacter nicotinovorans* MAHUQ-43 exhibited higher antibacterial activity against the Gram-negative *P. aeruginosa* (24.7 mm) than Gram-positive *B. cereus* (19.3 mm). In addition, Abd-Elnaby et al. (2016) biosynthesized Ag-NPs using the marine *Streptomyces rochei* MHM13; which exhibited high antibacterial efficacy against *B. subtilis*, *S. aureus*, *P. aeruginosa*, *B. cereus*, *S. typhimurium*, and *E. coli* with inhibition zone ranging 16-18 mm. The MIC and MBC of biosynthesized Ag-NPs were determined as an estimation of their antimicrobial activity against the Gram-positive *L. monocytogenes* and Gram-negative *K. pneumonia* as multidrug-resistant pathogens. The disc diffusion methods are attractive because of their simplicity and low cost, but, it could not always be accurate for screening the antimicrobial activity, especially for insoluble compounds as Ag-NPs (Klančnik et al. 2010). Results indicated that the Gram-negative *K. pneumonia* was more sensitive to Ag-NPs than the Gram-positive *L. monocytogenes*. Similarly, Klančnik et al. (2010) reported that MIC values generally obtained by the agar dilution method were 3-20 times lower than MIC obtained by disc diffusion.

Tolerance level and SEM imaging

To determine if the biosynthesized Ag-NPs have a bactericidal or bacteriostatic effect against the tested pathogenic strains, the tolerance level should be evaluated. When MBC/MIC ratio is ≥ 16 , the antimicrobial agent is considered bacteriostatic, and when the ratio is ≤ 4 , the agent is considered bactericidal (Das et al. 2017). The National Clinical Committee for Laboratory Standards reported that an agent is considered as bactericidal when it causes 99.9% reduction in CFU/mL after 18-24h of incubation in broth media, and if its MBC is higher than its MIC by 32-fold or more, the microbe is considered as tolerant (Woods and Washington 1995). The tolerance level of 2, for *L. monocytogenes*, and *K. pneumonia* confirmed that Ag-NPs biosynthesized by *S.* have a biocidal effect against both pathogens.

The morphological changes of *L. monocytogenes* and *K. pneumonia* treated with biosynthesized Ag-NPs were investigated by SEM. Cells treated with their MIC of biosynthesized Ag-NPs after 6 h showed notable morphological alterations as compared to the control suggesting the bactericidal effect of biosynthesized Ag-NPs (Sondi and Salopek-Sondi 2004). Likewise, Sondi and Salopek-Sondi (2004) reported that the antimicrobial activity of Ag-NPs against *E. coli*, is probably due to pits formation in the treated cell wall, leading to its death. The peptidoglycan protein layer in bacterial cell structure is negatively charged making more positively charged Ag^+ ions get stuck in Gram-positive than in Gram-negative bacteria. Gram-positive cell wall is thicker than that of Gram-negative bacteria, which slows down the passage of Ag-NPs through the cell wall and thus, allowing less Ag-NPs to reach the cytoplasmic membrane. As a result,

Gram-negative bacteria are more susceptible than Gram-positive ones (Ahmed et al. 2016; Erjaee et al. 2017). In addition, the uptake, accumulation, and translocation of the Ag-NPs inside the cells depend on the structure of cells, its permeability, and other cell properties, and also the size of NPs (Li et al. 2015). Tripathi et al. (2017) reported that Ag⁺ ions can accumulate in the microbial cytoplasm or near its cell wall, disrupting the membrane, and increasing its permeability thus, affecting proper transport, and facilitating the entrance of Ag-NPs. Furthermore, it is suggested that Ag-NPs bind to thiol groups (-SH) of enzymes forming stable S-Ag bonds with thiol containing compounds causing deactivation of the enzymes involved in transport (Ahmed et al. 2016). It was also proposed that Ag⁺ ion can enter the cell and disrupt the hydrogen bonding between DNA strands, denature it, thus DNA loses its replication capability and expression of ribosomal proteins (Shahverdi et al. 2007). Furthermore, Ag-NPs can enter the inner membrane which inhibit respiratory enzyme(s), facilitating the production of reactive oxygen species, and inhibiting the microbial growth (Das et al. 2017).

Cytotoxicity assessment

Cytotoxicity is an important factor that affect safety applications of Ag-NPs. The MTT assay is based on the capability of mitochondrial-dehydrogenase enzyme to cleave the tetrazolium rings of MTT and form dark-blue insoluble formazan crystals, that is highly impermeable, thus accumulates within living cells (Hansen et al. 1989). While, the SRB assay does not depend on the enzymatic activity, but, it is rather used to determine the cell density by measuring cellular protein content (Vichai and Kirtikara 2006).

Dermal contact is one of the most common ways of NPs exposure (Schneider et al. 2009). Therefore, it is proposed that NPs can be used in cosmetics if only its effect and absorption were evaluated on skin by the European Commission's Safety Committee (Kong et al. 2011). So, the use of standard HSF cell lines would be effective to screen *in vitro* cytotoxicity of the produced Ag-NPs. The lower toxicity observed on normal cell lines is probably due to the bioactive molecules present in the biological source used for biogenic synthesis and capping of biosynthesized Ag-NPs, which influenced the properties of the biosynthesized Ag-NPs (Roy et al. 2019).

The potential cytotoxicity of NPs depends on the routes of administration and NPs characteristics, such as size, shape, concentration, and time exposure as well as capping agent used for NPs stabilization (Niska et al. 2016; Tayel et al. 2017). The effect of different capping agents on cytotoxicity was investigated by Niska et al. (2016) using spherical-shaped Ag-NPs capped with lipoic acid (AgNPs-LA), polyethylene glycol (AgNPs-PEG), or tannic acid (AgNPs-TA) and compared with uncapped ones, against human gingival fibroblast cells using MTT assay. Capped-AgNPs-LA had the lowest cytotoxicity, followed by AgNPs-PEG, and AgNPs-TA. The differences in cytotoxicity between all capped Ag-NPs clearly reveal the influence of capping agents on Ag-NPs cytotoxicity. On the contrary, Wypij et al. (2018) reported that the IC₅₀ of biosynthesized Ag-NPs using *Streptomyces xinghaiensis* OF1 strain was 4 and 3.8 µg ml⁻¹ against mouse fibroblasts and cancer HeLa cell lines, respectively, with dose dependent potential.

Furthermore, Paknejadi et al. (2018) reported that chemically-prepared Ag-NPs exhibited higher cytotoxicity on normal HSF cell lines with IC₅₀ values of 30.64 and 14.98 µg/mL, after 24h and 48h of incubation, respectively. Similarly, Kummara et al. (2016) biosynthesized spherical Ag-NPs using *Azadirachta indica* leaves extract, and compared its cytotoxic effect with chemically synthesized Ag-NPs against lung cancer cell-line (NCI-H460) and Human Skin Dermal Fibroblast (HDFa). The biosynthesized Ag-NPs have higher cytotoxic effect on cancer cells than chemically synthesized ones. It is worth mentioning that cancerous cells were more likely sensitive to Ag-NPs than normal cells (Paknejadi et al. 2018). On the other hand, biosynthesized Ag-NPs treatment at different concentrations (0-240 µg/mL) didn't decrease the viability of normal HDFa cells and did not show any significant change even at higher concentration, while, chemically synthesized Ag-NPs showed a significant toxicity confirming that biosynthesized Ag-NPs were much safer on normal HDFa than chemically synthesized ones.

The biosynthesized Ag-NPs in this study had significantly higher IC₅₀ than its MBC values against the two studied multidrug-resistant pathogens (*K. pneumonia* and *L. monocytogenes*). Consequently, it is safer to be used as an effective antimicrobial agent in medical, cosmetics, and other industrial applications.

Genotoxicity assessment

One of the major issue in identification of Ag-NPs hazards is its genotoxic potential which is identified by the European Food Safety Authority (EFSA) as important step in safety evaluation in food and feed (EFSA Scientific Committee et al. 2016). Genotoxicity data provides a more comprehensive risk assessment of Ag-NPs. The comet assay is sharp, easy, and flexible assay used for the identification of DNA degradation in eukaryotic cells. The comet assay is often used in nano-genotoxicology to identify substances that can cause DNA damage (Glei et al. 2016). It enables the detection of cross-links, and disruption of double-stranded DNA (Sharma et al. 2020). Similarly, Narciso et al. (2020) reported no genotoxic damage by comet assay in selected tissues (blood, spleen, liver, kidney, duodenum) of male and female mice, when exposed orally to Ag-NPs of 50, 150, 300 mg/kg.wt/day for 3 days. In contrast, Patlolla et al. (2015) reported a significant increase in the genotoxic effects, such as chromosome aberrations, and damaged DNA in rats treated with Ag-NPs for five days, at doses of 5-100 mg/Kg.wt.day. Genotoxicity using comet assay provide new insight for complete risk assessment of Ag-NPs via oral route (Narciso et al. 2020). Collectively, different physicochemical characteristics, including size, shape, and administration route can lead to different biological activities, thus affecting the outcome of hazard identification (Tayel et al. 2017; Narciso et al. 2020).

Conclusion

Streptomyces roseolus was isolated from the Egyptian soil and molecularly identified using 16S rRNA gene sequencing. *S. roseolus* was efficiently used for the first time as simple, green, cost-effective bio-reducing agent for extracellular biosynthesis of Ag-NPs, after 6 h at room temperature. Characterization of Ag-NPs was evaluated using UV-Vis spectroscopy, TEM, DLS, FTIR, and XRD. FTIR analysis confirmed that the bio-reduction of Ag⁺ ions to Ag-NPs and its stabilization by different biomolecules in the *Streptomyces* supernatant, which probably influenced its biosafety. The MBC of the biosynthesized Ag-NPs against *L. monocytogenes* and *K. pneumonia* were 195 and 48 µg/mL, respectively, with tolerance level of 2 confirming its biocidal effect. SEM imaging showed complete destruction of pathogen cells after 6 h of exposure to MIC. The cytotoxic effect of biosynthesized Ag-NPs showed IC₅₀ of 8210 µg/mL for normal Blood Lymphocytes, and < 300 µg/mL for HSF normal cell-lines, assuring safe application as antiseptic and in dermal practice. No genotoxicity was recorded using comet assay with zero tail migration and 100% head intensity indicating no genotoxic effect of the biosynthesized Ag-NPs on Blood Lymphocytes. The biosynthesized Ag-NPs exhibited strong antimicrobial activity, with no harmful effect on different normal cell-lines, indicating their prospective for safe-usage in biomedical, cosmetics, and industrial applications.

Declarations

Conflict of Interest Statement

All authors declare that there are no financial/commercial conflicts of interest.

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Contributions

Conceptualization, writing, editing, and supervision: Noha Sorour and Rateb abbas.

Laboratory work, and data analysis: Asmaa Elnady, Rateb abbas

All authors read and approved the final manuscript.

Supplementary data are available upon request.

References

1. Abd-Elnaby HM, Abo-Elala GM, Abdel-Raouf UM, Hamed MM (2016) Antibacterial and anticancer activity of extracellular synthesized silver nanoparticles from marine *Streptomyces rochei* MHM13. *The Egyptian Journal of Aquatic Research* 42(3):301–312
2. Ahmed S, Ahmad M, Swami BL, Ikram S (2016) A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. *Journal of advanced research* 7:17–28
3. Alsamhary KI (2020) Eco-friendly synthesis of silver nanoparticles by *Bacillus subtilis* and their antibacterial activity. *Saudi Journal of Biological Sciences* 27:2185–2191
4. Anandaradje A, Meyappan V, Kumar I, Sakthivel N (2020) Microbial synthesis of silver nanoparticles and their biological potential. *Nanoparticles in Medicine*, p. 99–133. Springer
5. Andrews JM (2001) Determination of minimum inhibitory concentrations. *Journal of antimicrobial Chemotherapy* 48:5–16
6. Bakhtiari-Sardari A, Mashreghi M, Eshghi H, Behnam-Rasouli F, Lashani E, Shahnava B (2020) Comparative evaluation of silver nanoparticles biosynthesis by two cold-tolerant *Streptomyces* strains and their biological activities. *Biotech Lett* 42:1985–1999
7. Crisan CM, Mocan T, Manolea M, Lasca LI, Tăbăran F-A, Mocan L (2021) Review on silver nanoparticles as a novel class of antibacterial solutions. *Applied Sciences* 11:1120
8. Das B, Dash SK, Mandal D, Ghosh T, Chattopadhyay S, Tripathy S, Das S, Dey SK, Das D, Roy S (2017) Green synthesized silver nanoparticles destroy multidrug resistant bacteria via reactive oxygen species mediated membrane damage. *Arabian Journal of Chemistry* 10:862–876
9. EFSA Panel on Food Additives and Nutrient Sources added to Food (2016) Scientific opinion on the re-evaluation of silver (E 174) as food additive. *EFSA Journal* 14:1:4364
10. El-Bendary MA, Afifi SS, Moharam ME, Abo El-Ola SM, Salama A, Omara EA, Shaheen MN, Hamed AA, Gawdat NA (2021) Biosynthesis of silver nanoparticles using isolated *Bacillus subtilis*: characterization, antimicrobial activity, cytotoxicity, and their performance as antimicrobial agent for textile materials. *Preparative Biochemistry Biotechnology* 51:54–68

11. Erjaee H, Rajaian H, Nazifi S (2017) Synthesis and characterization of novel silver nanoparticles using Chamaemelum nobile extract for antibacterial application. *Adv Nat Sci NanoSci NanoTechnol* 8:025004
12. Fissan H, Ristig S, Kaminski H, Asbach C, Epple M (2014) Comparison of different characterization methods for nanoparticle dispersions before and after aerosolization. *Anal Methods* 6:7324–7334
13. Garg D, Sarkar A, Chand P, Bansal P, Gola D, Sharma S, Khantwal S, Mehrotra R, Chauhan N, Bharti RK (2020) Synthesis of silver nanoparticles utilizing various biological systems: mechanisms and applications—a review. *Progress in Biomaterials* 1–15
14. Ghaseminezhad SM, Hamed S, Shojaosadati SA (2012) Green synthesis of silver nanoparticles by a novel method: Comparative study of their properties. *Carbohydrate polymers* 89:467–472
15. Glei M, Schneider T, Schlörmann W (2016) Comet assay: an essential tool in toxicological research. *Arch Toxicol* 90:10:2315–2336
16. Hansen MB, Nielsen SE, Berg K (1989) Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. *J Immunol Methods* 119:203–210
17. Hu D, Chen Y, Sun C, Jin T, Fan G, Liao Q, Mok KM, Lee M-YS (2018) Genome guided investigation of antibiotics producing actinomycetales strain isolated from a Macau mangrove ecosystem. *Scientific reports* 8:1–12
18. Huq M, Akter S (2021) Bacterial Mediated Rapid and Facile Synthesis of Silver Nanoparticles and Their Antimicrobial Efficacy against Pathogenic Microorganisms. *Materials* 14:2615
19. Ibrahim HM (2015) Green synthesis and characterization of silver nanoparticles using banana peel extract and their antimicrobial activity against representative microorganisms. *Journal of radiation research applied sciences* 8:265–275
20. Kannan R, Stirk W, Van Staden J (2013) Synthesis of silver nanoparticles using the seaweed *Codium capitatum* PC Silva (Chlorophyceae). *South African Journal of Botany* 86:1–4
21. Kim M, Morrison M, Yu Z (2011) Evaluation of different partial 16S rRNA gene sequence regions for phylogenetic analysis of microbiomes. *J Microbiol Methods* 84:81–87
22. Kizhakeyil A, Ong ST, Fazil MHUT, Chalasani MLS, Prasannan P, Verma NK (2019) Isolation of human peripheral blood T-lymphocytes. *T-Cell Motility* 11–17. Springer
23. Klančnik A, Piskernik S, Jeršek B, Možina SS (2010) Evaluation of diffusion and dilution methods to determine the antibacterial activity of plant extracts. *J Microbiol Methods* 81:121–126
24. Kong B, Seog JH, Graham LM, Lee SB (2011) Experimental considerations on the cytotoxicity of nanoparticles. *Nanomedicine* 6:929–941
25. Kummara S, Patil MB, Uriah T (2016) Synthesis, characterization, biocompatible and anticancer activity of green and chemically synthesized silver nanoparticles—a comparative study. *Biomedicine Pharmacotherapy* 84:10–21
26. Li X, Schirmer K, Bernard L, Sigg L, Pillai S, Behra R (2015) Silver nanoparticle toxicity and association with the alga *Euglena gracilis*. *Environmental Science: Nano* 2:594–602
27. Loo YY, Rukayadi Y, Nor-Khaizura MA, Kuan CH, Chieng BW, Nishibuchi M, Radu S (2018) In vitro antimicrobial activity of green synthesized silver nanoparticles against selected gram-negative foodborne pathogens. *Frontiers in microbiology* 9:1555
28. Malarkodi C, Rajeshkumar S, Paulkumar K, Vanaja M, Jobitha GDG, Annadurai G (2013) Bactericidal activity of bio mediated silver nanoparticles synthesized by *Serratia nematodiphila*. *Drug invention today* 5:119–125
29. Martínez G, Merinero M, Pérez-Aranda M, Pérez-Soriano EM, Ortiz T, Begines B, Alcudia A (2021) Environmental impact of nanoparticles' application as an emerging technology: A review. *Materials* 14:166

30. May J, Shannon K, King A, French G (1998) Glycopeptide tolerance in *Staphylococcus aureus*. *J Antimicrob Chemother* 42:189–197
31. Mourdikoudis S, Pallares RM, Thanh NT (2018) Characterization techniques for nanoparticles: comparison and complementarity upon studying nanoparticle properties. *Nanoscale* 10:12871–12934
32. Narciso L, Coppola L, Lori G, Andreoli C, Zjino A, Bocca B, Petrucci F, Di Virgilio A, Martinelli A, Tinari A, Maranghi F (2020) Genotoxicity, biodistribution and toxic effects of silver nanoparticles after in vivo acute oral administration. *NanoImpact* 1:18:100221
33. Niska K, Knap N, Kędzia A, Jaskiewicz M, Kamysz W, Inkielewicz-Stepniak I (2016) Capping agent-dependent toxicity and antimicrobial activity of silver nanoparticles: an in vitro study. Concerns about potential application in dental practice. *International journal of medical sciences* 13:772
34. Paknejadi M, Bayat M, Salimi M, Razavilar V (2018) Concentration-and time-dependent cytotoxicity of silver nanoparticles on normal human skin fibroblast cell line. *Iran Red Crescent Med J* 20
35. Patlolla AK, Hackett D, Tchounwou PB (2015) Silver nanoparticle-induced oxidative stress-dependent toxicity in Sprague-Dawley rats. *Molecular and cellular biochemistry* 1:257 – 68
36. Roy A, Bulut O, Some S, Mandal AK, Yilmaz MD (2019) Green synthesis of silver nanoparticles: biomolecule-nanoparticle organizations targeting antimicrobial activity. *RSC Adv* 9:2673–2702
37. Schneider M, Stracke F, Hansen S, Schaefer UF (2009) Nanoparticles and their interactions with the dermal barrier. *Dermato-endocrinology* 1:197–206
38. Shahverdi AR, Fakhimi A, Shahverdi HR, Minaian S (2007) Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against *Staphylococcus aureus* and *Escherichia coli*. *Nanomed Nanotechnol Biol Med* 3:168–171
39. Sharma D, Shandilya P, Saini NK, Singh P, Thakur VK, Saini RV, Mittal D, Chandan G, Saini V, Saini AK (2021) Insights into the synthesis and mechanism of green synthesized antimicrobial nanoparticles, answer to the multidrug resistance. *Materials Today Chemistry* 1:19:100391
40. Sharma V, Kaushik S, Pandit P, Dhull D, Yadav JP, Kaushik S (2019) Green synthesis of silver nanoparticles from medicinal plants and evaluation of their antiviral potential against chikungunya virus. *Appl Microbiol Biotechnol* 103:881–891
41. Singh NP, McCoy MT, Tice RR, Schneider EL (1988) A simple technique for quantitation of low levels of DNA damage in individual cells. *Experimental cell research* 175:1:184–191
42. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR (1990) New colorimetric cytotoxicity assay for anticancer-drug screening. *JNCI: Journal of the National Cancer Institute* 82:1107–1112
43. Sondi I, Salopek-Sondi B (2004) Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria. *J Colloid Interface Sci* 275:177–182
44. Srikar SK, Giri DD, Pal DB, Mishra PK, Upadhyay SN (2016) Green synthesis of silver nanoparticles: a review. *Green Sustainable Chemistry* 6:34–56
45. Sumadevi K, Krishnamurthy G, Walmik P, Rani RP, Naik S, Naik HB, Naik N (2021) Photocatalytic degradation of Eriochrome black-T and Evan's blue dyes under the visible light using PVA capped and uncapped Ag doped ZnS nanoparticles. *Emergent Materials* 4:447–456
46. Tamboli DP, Lee DS (2013) Mechanistic antimicrobial approach of extracellularly synthesized silver nanoparticles against gram positive and gram negative bacteria. *J Hazard Mater* 260:878–884
47. Tayel AA, Sorour NM, El-Baz AF, Wael F (2017) Nanometals appraisal in food preservation and food-related activities. *Food Preservation* 487–526. Elsevier

48. Tripathi DK, Tripathi A, Singh S, Singh Y, Vishwakarma K, Yadav G, Sharma S, Singh VK, Mishra RK, Upadhyay R (2017) Uptake, accumulation and toxicity of silver nanoparticle in autotrophic plants, and heterotrophic microbes: a concentric review. *Frontiers in microbiology* 8:7
49. Vanlalveni C, Lallianrawna S, Biswas A, Selvaraj M, Changmai B, Rokhum SL (2021) Green synthesis of silver nanoparticles using plant extracts and their antimicrobial activities: A review of recent literature. *RSC Advances* 11:2804–2837
50. Vichai V, Kirtikara K (2006) Sulforhodamine B colorimetric assay for cytotoxicity screening. *Nature protocols* 1:1112–1116
51. Wikler MA (2006) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard. *CLSI (NCCLS)* 26: M7-A7
52. Woods G, Washington J (1995) The clinician and the microbiology laboratory. *Principles and practice of infectious diseases, 4th ed Churchill Livingstone, Philadelphia* 169–199
53. Wypij M, Czarnecka J, Świecimska M, Dahm H, Rai M, Golinska P (2018) Synthesis, characterization and evaluation of antimicrobial and cytotoxic activities of biogenic silver nanoparticles synthesized from *Streptomyces xinghaiensis* OF1 strain. *World Journal of Microbiology Biotechnology* 34(2):1–3
54. Yaqoob AA, Umar K, Ibrahim MNM (2020) Silver nanoparticles: various methods of synthesis, size affecting factors and their potential applications—a review. *Applied Nanoscience* 10:1369–1378
55. Zhang X-F, Liu Z-G, Shen W, Gurunathan S (2016) Silver nanoparticles: synthesis, characterization, properties, applications, and therapeutic approaches. *Int J Mol Sci* 17:1534

Tables

Table (1). Antimicrobial activity of Ag-NPs biosynthesized by *S. roseolus* against different multidrug-resistant strains, measured as inhibition zone diameter in mm.

Zone of Inhibition (mm)								
Gram-positive strains				Gram-negative strains				Yeast
Conc. (mg/mL)	<i>L. monocytogenes</i>	<i>B. cereus</i>	<i>B. subtilis</i>	<i>S. aureus</i>	<i>Aeromonas hydrophilia</i>	<i>E. coli</i> O157:H7	<i>K. pneumonia</i>	<i>C. albicans</i>
25	20 ± 0	20 ± 0.3	19.66 ± 0.3	20.66 ± 0.3	21 ± 0.3	23.66 ± 0	22 ± 0.3	18.66 ± 0.3
12.5	18 ± 0.3	18.5 ± 0.3	17 ± 0	18.66 ± 0.3	19 ± 0.3	22.66 ± 0	19 ± 0.3	17.66 ± 0.3
6.25	16.66 ± 0.3	16.33 ± 0.3	15.66 ± 0.3	17.33 ± 0.3	16.5 ± 0.5	17.33 ± 0.4	17.5 ± 0.3	15 ± 0
3.125	14.33 ± 0.3	14.33 ± 0.3	13.66 ± 0.3	15.33 ± 0.3	14.5 ± 0.5	18.33 ± 0.4	15 ± 0.3	13 ± 0.3
1.56	11.66 ± 0.3	11.66 ± 0.3	11.66 ± 0.3	14 ± 0.5	12 ± 0.5	15.66 ± 0	13 ± 0	12.5 ± 0.3
0.78	9.66 ± 0.3	9.66 ± 0.3	8.66 ± 0.3	12.33 ± 0.3	10.5 ± 0.3	13 ± 0.3	10.5 ± 0	9.66 ± 0.3
0.39	7.33 ± 0.3	7.33 ± 0.3	6.33 ± 0.3	10.33 ± 0.3	8.5 ± 0	10 ± 0	8.5 ± 0.3	6.66 ± 0.3
AgNO ₃	5 ± 0.3	5 ± 0.3	4 ± 0.3	6 ± 0.5	5 ± 0.3	6 ± 0.3	5 ± 0	5 ± 0.5
<i>S. roseolus</i> supernatant	0	0	0	0	0	0	0	0
Data are average d of 3 replicates ± SD (standard deviation).								

Table (2). The antibacterial activity of biosynthesized Ag-NPs determined as MIC and MBC using agar dilution and broth macro-dilution methods.

Concentration (µg/mL)	<i>Listeria monocytogenes</i>		<i>Klebsiella pneumonia</i>	
	Agar dilution	Broth macro-dilution	Agar dilution	Broth macro-dilution
MIC	195	97	48	24
MBC	195	195	97	48
Tolerance level (MBC/MIC)	1	2	2	2
MBC/MIC ratio ≥ 16, the antimicrobial agent is considered bacteriostatic, and MBC/MIC ratio ≤ 4, the agent is considered bactericidal.				

Table (3). Mean value of cell viability of normal and HepG cell-lines (% of control) ± S.D at different concentrations of biosynthesized Ag-NPs. IC₅₀ was calculated in µg/mL.

Cell line	Concentration	Viability mean	Standard deviation	IC ₅₀ (µg/mL)
Human Skin Fibroblast	Control	100	0	
	0.03	98.90	1.69	
	0.3	94.52	0.48	> 300
	3	91.12	1.24	
	30	89.13	0.054	
	300	55.85	0.42	
Hepatocellular Carcinoma	500	69.16	0.63	
	250	72.59	1.25	1147
	125	75.15	2.73	
	62.5	83.60	4.75	
Peripheral Blood Lymphocytes	500	97.64	2.6	
	195	88.54	14.5	8210
	97.5	99.57	1.54	
	48	92.95	8.9	

Figures

Figure 1

Streptomyces roseolus grown on starch nitrate agar medium (A), under light microscope *100 (B), UV-Vis Scanning of biosynthesized Ag-NPs by *S. roseolus* culture supernatant at different temperature (C), and XRD analysis of biosynthesized Ag-NPs (D).

Figure 2

Phylogenetic tree of 16S sequences of the Streptomyces isolate #140 aligned with closely related sequences accessed from the GenBank under accession no. MT071505.

Figure 3

TEM analysis of Ag-NPs biosynthesized by *S. roseolus* culture supernatant, and hydro-dynamic size determination of biosynthesized Ag-NPs by *S. roseolus* using DLS.

Peak	Group frequency (cm ⁻¹)	Functional group assignment
1	3425.45	NH (primary amine stretch)
2	2952.28	CH (alkane stretch)
3	2159.91	O, C, O (CO ₂ stretch)
4	1614	C=C (alkenyl stretch)
5	1430.17-1370	C-H or CH ₂ (alkanes)
6	1154.34	C--O (carboxylic acid)
7	1079-1025.24	C-N, primary amine, (CN stretch)
8	707.26	Thiol or thioether, CH ₂ -S- (C-S stretch)
9	700-600	Aliphatic chloro-compounds, (C-Cl stretch)

Figure 4

FTIR analysis of Ag-NPs biosynthesized by *Streptomyces roseolus*.

Figure 5

Antimicrobial activity of biosynthesized Ag-NPs was tested using disc diffusion method by two-fold dilution against *L. monocytogenes* (A), *S. aureus* (B), *B. subtilis* (C), *B. cereus* (D), *E. coli* O157:H7 (E), *A. hydrophilia* (F), *K. pneumonia* (G), and *C. albicans* (H).

Figure 6

SEM micrograph showing the antimicrobial action of biosynthesized Ag-NPs against *L. monocytogenes* (A), and *K. pneumoniae* (B) at MIC concentration after 3h (C, D) and 6h of incubation (E, F).

Figure 7

Cytotoxic effect of biosynthesized Ag-NPs against Hep-G2 (A), lymphocytes (B) using MTT assay (n=3), and HSF (C), using SRB assay, data expressed as the mean value of cell viability (% of control) $\pm$ S.D. IC50 was calculated to be 1147 μg/mL, 8210 μg/mL, and <300 μg/mL, respectively.

Figure 8

Single cell gel electrophoresis comet analysis of untreated (A) and peripheral Blood Lymphocytes (B, C, D) treated with different concentration of the biosynthesized Ag-NPs

Supplementary Files

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