Original Article

Antibacterial and Antioxidant Potential of Silver Nanoparticles Bio-Friendly Synthesis Using *Delftia acidovorans* (OM838393.1) Strain

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Abstract

Background: The burgeoning field of nanotechnology has witnessed a surge in the biological synthesis of nanoparticles, primarily due to their multiple applications in various domains. **Objectives:** This study delves into the biosynthesis of silver nanoparticles (AgNPs) utilizing the bacterium *Delftia acidovorans* (OM838393.1) strain and then determines their anti-MDR bacterial and antioxidant activity (*in vitro*) by DPPH assay. **Materials and Methods:** Bio-synthesis of AgNPs from *Delftia acidovorans* (OM838393.1) strain, characterized by using UV-visible spectroscopy, scanning electron microscopy (SEM), and X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR analysis) and determination their antibacterial and antioxidant activity. **Results:** UV-visible revealed a peak of 480 nm, crystalline nature of the biosynthesized nanoparticles with an average size of 69.7 nm scale with spherical shape. The synthesized AgNPs showed antibacterial efficacy against clinical bacterial isolates of MDR in both Gram-negative, *Streptococcus pneumoniae,* and Gram-positive, *Pseudomonas aeroginosa.* By using the disk diffusion method. This activity might be attributed to the unique biological and physicochemical properties of the AgNPs, which facilitate the disruption of bacterial cell membranes, from results underscore the potential of *Delftia acidovorans* (OM838393.1) strain as an eco-friendly and efficient biological agent for the synthesis of AgNPs with potent antibacterial properties. The produced biosynthetic AgNPs revealed a significantly high-antioxidant activity compared to ascorbic acid in the same concentration. **Conclusion:** It can be concluded that AgNPs can be bio-synthesized using *Delftia acidovorans* (OM838393.1) strain bacteria in an eco-friendly way and these AgNPs can be used as a cost-effective antibacterial agent and antioxidant.

Keywords: AgNPs, anti-microbial and antioxidant, biosynthetic NPs, Delftia acidovorans (OM838393.1) strain

Introduction

The realm of nanotechnology has witnessed a paradigm shift with the advent of biologically synthesized nanoparticles. These nanoparticles, particularly silver nanoparticles (AgNPs), have variable significant attention due to their broad-spectrum antibacterial activity and potent radical scavenging capacity.[1] Among various biological agents employed in the biosynthesis of AgNPs, microorganisms have emerged as potential candidates due to their eco-friendly, cost-effective, and scalable production.[2] A prominent bacterium in this domain is *Delftia acidovorans*, a Gram-negative bacterium renowned for its silver resistance and capability to bio-reduce silver ions into nanoparticles. The mechanism underlying the biosynthesis of AgNPs via *Delftia acidovorans* entails the enzymatic reduction of silver ions. The bacteria secrete molecules that facilitate bio-reduction, leading to the formation of stable nanoparticles with distinct morphologies.[3] Furthermore, these biologically synthesized AgNPs showcase enhanced antibacterial activity against a myriad of pathogens, including antibiotic-resistant

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strains. The nanoparticles exert their antibacterial effects by disrupting the bacterial cell wall, interfering with DNA replication, and promoting reactive oxygen species generation.[4] Moreover, these AgNPs also display a remarkable scavenging capacity, neutralizing free radicals and thereby offering protection against oxidative stress-induced cellular damage.[5] Such antioxidant potential positions them as suitable candidates in various medical and industrial applications, ranging from wound healing to water purification.

In this study, a novel bacterial isolate, *Delftia acidovorans* (OM838393.1) strain, was able to synthesize AgNPs as extracellular synthesise. The characterization of AgNP synthesize was investigated by UV-visible, XRD, FTIR, and SEMEDS, and then antimicrobial and anti-oxidants of AgNP activity were studied.

Materials and Methods

Bacteria *Delftia acidovorans* (OM838393.1) strain isolate was obtained from the hospital floor. Diagnosis in Microbiology Lab, Biology Department, College of Science, University of Babylon, Iraq,[6] and confirmed diagnosis of bacteria was done according to Clinical and Laboratory Standards Institute (CLSI).[7]

Solution and media

Silver nitrate salt ($AgNO_3$), brain heart infusion agar and broth medium, Mueller-Hinton agar, antibiotics disc, ascorbic acid, and other chemical solutions and reagents were purchased from Merck- Germany.

Biosynthesis of silver nanoparticles by *Delftia acidovorans* (OM838393.1) strain

The biosynthesis of silver nanoparticles was performed by Kumar *et al.*[8] with some modifications. Bacteria were cultured in Luriae-Bertanie (LB) broth at 37°C and 200 rpm for, 24 h. Bacterial cells were harvested by centrifugation at 10,000/rpm, for 10 min, and washed with sterile distilled water. Bacterial cells were resuspended in 50 mM phosphate buffer (pH 7.0) and adjusted the optical density to 0.6 at 600 nm. 1 mM of silver nitrate ($AgNO_3$) colorless solution was added to the yellow bacterial suspension and incubated at room temperature under dark conditions for 24 h, then the clay-brown color of AgNPs was formed [Figure 1]. AgNPs were collected by centrifugation at 14,000 rpm for 30 min and filtration through 0.22 µm membrane filter. AgNPs were rewashed three times by ethyl alcohol and precipitated by cooling centrifuge at 14,000 rpm for 30 min. The detection of AgNPs by the UV–Vis diffuse reflectance measurements, X-ray diffraction measurements (XRD), Fourier transform infrared (FTIR), and measurements of the field emission-scanning electron microscopes (FE-SEM).

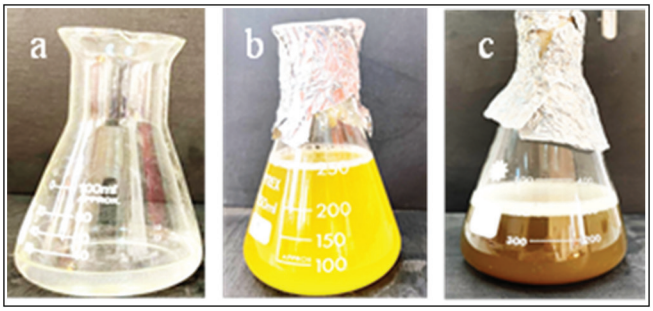

Figure 1: (A–C). $AgNO_3$ 1mM solution (A), Broth of *Delftia acidovorans* (OM838393.1) strain (B) and AgNPs formation as a positive result: brownish color (C)

Anti-bacterial activity of AgNPs against multi-drug resistant pathogenic bacteria

Anti-bacterial activity of AgNPs bio-synthesized by *Delftia acidovorans* (OM838393.1) strain were used to; evaluate their ability to inhibit the growth of MDR bacteria under study (*Streptococcus pneumonia* and *Pseudomonas aeroginosa*).

Antibiotic susceptibility

Each isolate of identified bacterial was studied their antibiotic profiling against, 5 anti-biotic disks, cephalothin (KF-30 µg), methicillin (ME-5 µg), and novobiocin (NV-5 µg), doxycycline (DO-30 µg) and clarithromycin (CLR-15 µg) were used versus 31.25, 62.5, 125, 250, and 500 µg/mL of AgNPs. All tests were conducted using the Kirby-Bauer disk-diffusion method in plates containing Mueller-Hinton agar (Carl Roth, Germany). Zones of inhibition around each disc were assessed by computer-associated electronic zone analyzer in single-disc antimicrobial susceptibility testing in accordance with CLSI guidelines before each identified bacterial isolate was re-suspended to compared with 0.5 McFarland as standard turbidity (equivalent to 1.5×10^8 colony forming unite per milliliter [cfu/mL]).

Antibacterial properties of AgNPs

AgNP antibacterial abilities were tested against a number of human pathogens that were grown on nutrient agar slants. While evaluating the antibiotic activity of AgNPs, the guidelines provided by the Clinical and Laboratory Standards Institute were adhered to CLSI, 2012. Using a disk diffusion experiment, triplicates are employed in dilutions of AgNP concentrations (500, 250, 125, 62.5, and 31.25 µg/mL) in solvent to assess antibiotic sensitivity and AgNPs against the study microorganisms. In the first step, the isolates were incubated for 15 min at room temperature, then incubated at 37°C overnight when treated bacterial isolates with AgNPs against bacterial isolates under study. A digital Vernier caliper is used to measure the inhibitory zone's breathe after an incubation period during which the inhibition zone could be visible around the well (CLSI, 2016).

Free radical scavenging assay

Free radical scavenging activity by the DPPH (1,1-diphenyl, 2-picryl-hydrazine) procedure described by Kumar *et al.*[8] was used to determine the activity of silver nanoparticles synthesis by *Delftia acidovans* (OM838393.1) strain. The radical scavenging behavior of samples against the stable DPPH radical was determined spectrophotometrically using the ELISA reader using different concentrations of silver nanoparticles (12.5, 25, 50, 100, and 200 µg/ml). when the DPPH reduction was tested at 517 nm, the colorimetric transition (from deep violet to light yellow). Positive controls, such as ascorbic acid, have been used. The equation below has been used to calculate the inhibition percentage.

$$\text{Inhibition\%} = \frac{\text{Absorbace of negative control} - \text{Absorbance of sample}}{\text{Absorbance of negative control}} \times 100$$

Ethical approval

Valid consent was achieved from each patient who had taken a sample of bacteria before their inclusion in the study. All tested, the procedure the DNA Research Center and Microbiology Lab Biology Department, College of Science, University of Babylon had been informed before the samples were collected and conducted.

Results

Description of the synthesized silver nanoparticles

UV/ Vis spectrophotometer

The presence of AgNP nanoparticles was confirmed by obtaining a spectrum in the visible range of 300–800 nm using UV-visible spectrophotometer [Figure 2].

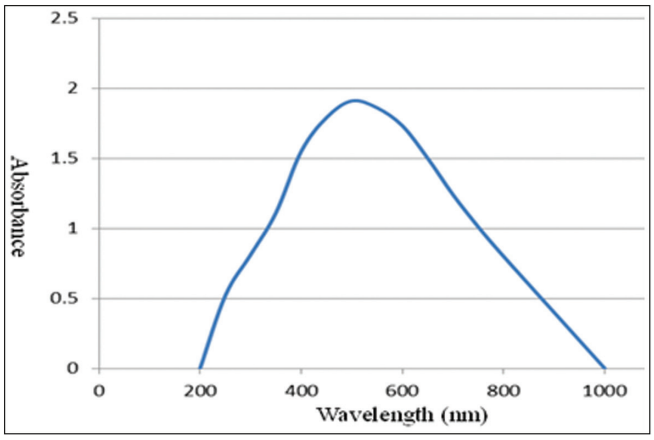

Figure 2: UV-visible spectra of the biosynthesized silver nanoparticles

Field emission-scanning electron microscopy (FE-SEM)

FE-SEM images revealed that the silver nanoparticles produced by the *Delftia acidovorans* (OM838393.1) strain bacteria tend to be spherical in shape with an average scale of 69.7 nm in size [Figure 3].

X-ray diffraction (XRD Examination)

Synthesized AgNPs crystal structure measured by using XRD technique by using (6000 XRD), at a step, with a voltage (40 KV) and electric current (30 mA), scanning rate of 2° with (2θ/min) and range of 2θ (10°–80°). Figure 4 shows a pattern of XRD of bio-synthesized Ag nanoparticles observed the peaks at showing 4 sharp peaks corresponding to the diffraction from 111, 200, 220 and 311 planes of AgNPs are metallic and have a crystal structure.

Fourier transform infrared spectroscopy (FTIR Analysis)

FT-IR spectrum samples were created using the (400–4000 cm^{-1}) SHIMADZU instrument. *Delftia acidovorans'* (OM838393.1) strain spectrum exhibits a strong peak at 3490 cm^{1}, which can be used to identify the functional group in samples [Figure 5]. This result denotes increased stretching of the amino group's O-H bond in alcohols and phenols and due to present hydroxyl, 3398.34, 3365.55, 3336.62, 3301.91, 3284.55, 3176.54, and 3114.82 cm^{-1} (N-H stretch) in different compounds such as primary amines, amides or other compounds, 2356.85, 1220.86, 1137.92, 1068.49 cm^{-1} (C-N stretch) in amines or amides, 628.75, 594.03 cm^{-1} stretching vibrations in thioether functional groups

Antimicrobial activity of AgNPs

The antimicrobial activity of different concentrations of silver nanoparticles (AgNPs) against *S. epidermidis* and *P. aeruginosa* are shown in Figures 6 and 7. This activity is compared with that of various antibiotics. The antimicrobial activity of AgNPs against both bacterial isolates increased with the increased concentration.

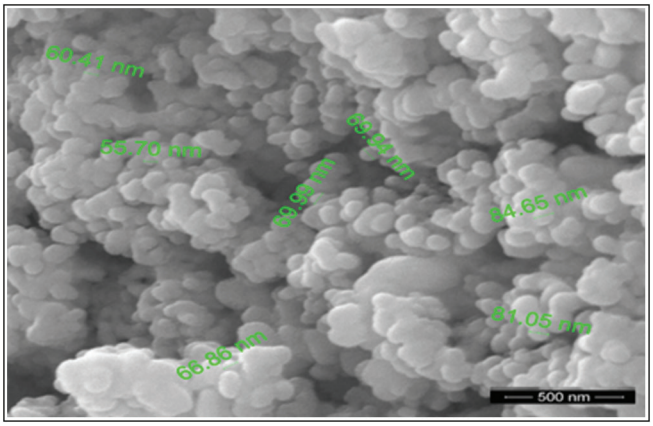

Figure 3: FE-SEM of AgNPs synthesized by *Delftia acidovorans* (OM838393.1) strain

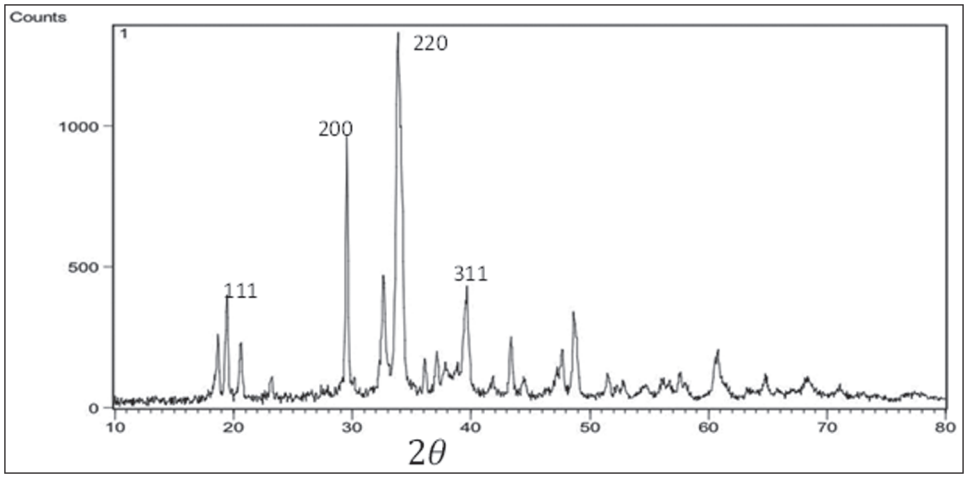

Figure 4: X-ray diffraction results for AgNPs synthesized by the reaction of $AgNO_3$ solution with *Delftia acidovorans* (OM838393.1) strain

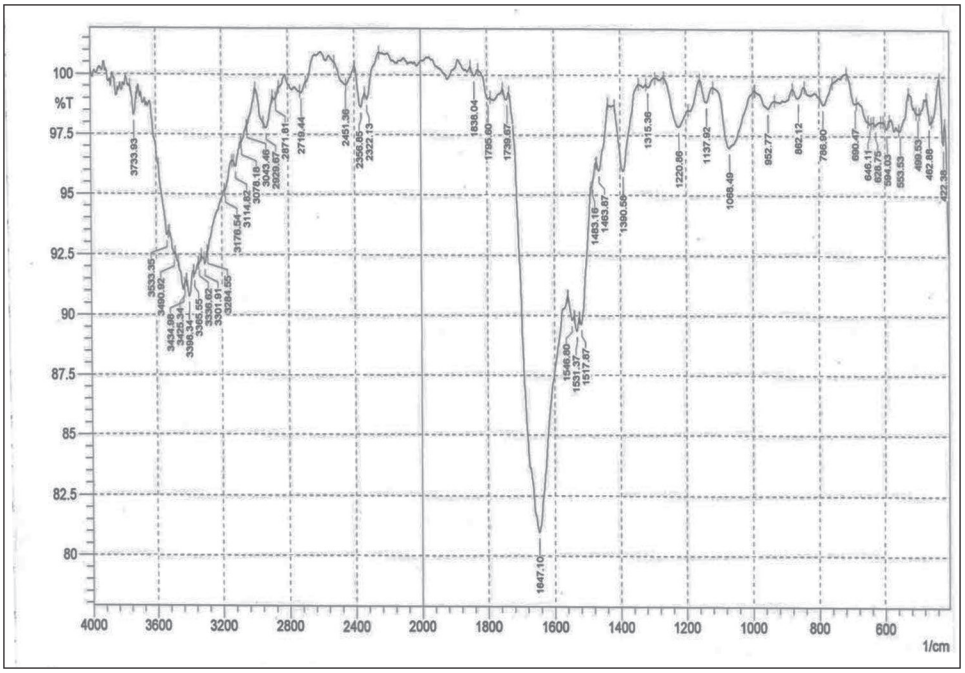

Figure 5: FTIR spectra pattern of AgNPs synthesized by the reaction of $AgNO_3$ solution with *Delftia acidovorans* (OM838393.1) strain

S. epidermidis and *P. aeruginosa* both show susceptibility to AgNPs, with *P. aeruginosa* showing slightly higher susceptibility at higher concentrations (AgNPs-500 and AgNPs-250). On the other hand, Cephalothin (KF-30), Methicillin (ME-5), and Novobiocin (NV-5) showed resistant activity against two tested bacterial isolates. Doxycycline (DO-30) exhibits significant activity against *S. epidermidis* but lesser activity against *P. aeruginosa*. Clarithromycin (CLR-15) shows moderate activity against both bacterial strains, with identical inhibition zones of 10 and 13 mm for *S. epidermidis* and *P. aeruginosa*, respectively. The data suggests that AgNPs can be a potent antimicrobial agent, potentially even more effective than some conventional antibiotics against the tested bacterial isolates.

Free radical scavenging activity assay

Concentrations of AgNPs used 12.5, 25, 50, 100, and 200 µg/mL demonstrated the highest antioxidant activity (70.76% at 200 µg/mL), whereas the concentrations of AgNPs used 100, 50, 25, and 12.5 µg/mL demonstrated antioxidant activity (64.66, 52.62, 40.36, and 29.36%), respectively. Ascorbic acid's antioxidant activity at the same concentration was (82.06, 74.81, 52.62, 40.36, and 29.36%), respectively [Figure 8]. The Student's *t* test revealed non-significant differences at 100, and 12.5 µg/mL concentrations while at 200, 50, and 25 µg/mL concentrations there were significant differences between AgNPs and ascorbic acid, however, the previous results revealed a promising antioxidant activity of AgNPs that biosynthesized by *Delftia acidovorans* (OM838393.1) strain bacteria.

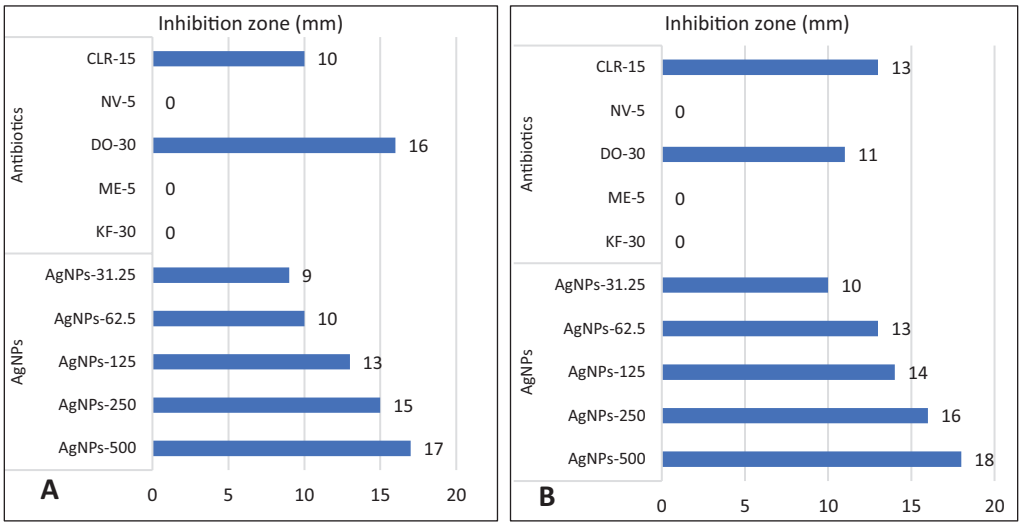

Figure 6: Antibacterial effect of different concentrations of AgNPs biosynthesis by *D. acidovorans* vs different traditional antibiotics against *S. epidermidis* (A), and *P. aeruginosa* (B)

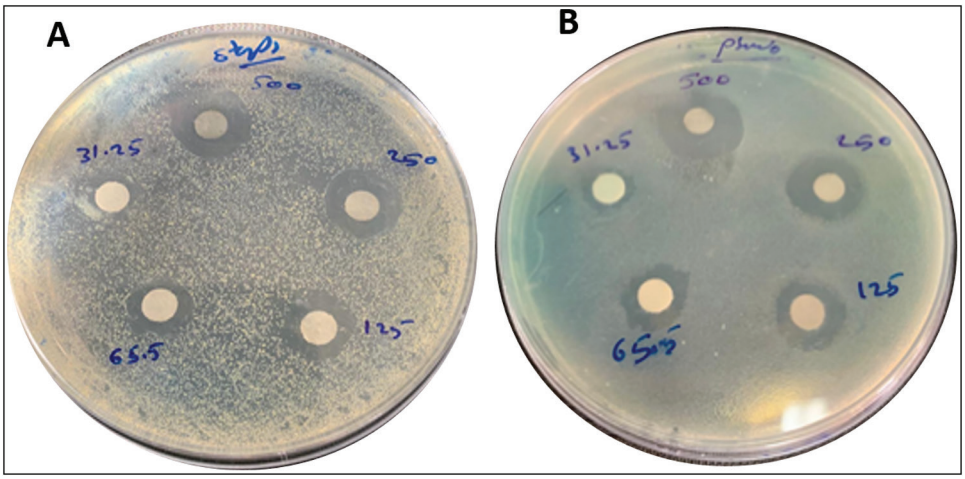

Figure 7: Sensitivity test of AgNPs different concentrations on Gram-positive *S. pneumoniae* (A) and Gram-negative *P. aeroginosa*. (B) cultured on Muller-Hinton agar

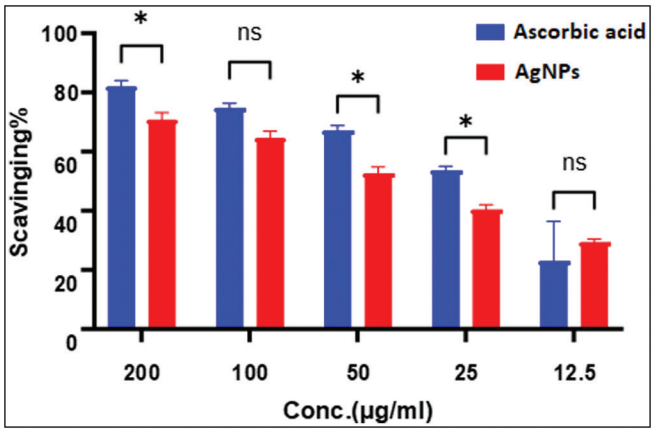

Figure 8: Free radical scavenging activity (%) of AgNPs different concentrations compared to Ascorbic acid as a positive control

Discussion

Description of the synthesized silver nanoparticles

UV/ Vis spectrophotometer

From this analysis, an absorbance peak was found at around 480 nm, which was specific for Ag nanoparticles that can be detected by changing the color of the mixture gradually from colorless to yellow to dark brownish after a few minutes from the beginning of the reaction in the solution and confirmed by UV-visible spectra [Figure 2]. The color change occurs when the number of particles increases, in the case of gold it is from deep red to purple. The varying color changes are due to the LSPR that they exhibit, and they lie in the visible region of the electromagnetic spectrum, which means that a particular portion of the wavelength in the visible region is absorbed while another

portion gets reflected and the emitted wavelength will reflect its own color. The absorbance of these color changes is measured using UV-visible spectroscopy[9] Many authors studied the effect of different bacterial strains to produce silver nanoparticles;[10] strong peaks at 420–480 nm due to the surface plasmon resonance UV-visible absorption spectrum of silver nanoparticles of three bacteria strains isolated from the soil; *Exiguobacterium aurantiacum*, *Escherichia coli* and *Brevundimonas diminuta*. The size of biosynthetic AgNPs varied with the variation of plant extraction concentrations.[11,12]

Field emission-scanning electron microscopy (FE-SEM)

From previous figures, the silver nanoparticles were found to be in various shapes like the nanorods, nonspherical, nanotriangles, and the different average diameters (1–100 nm) of AgNP synthesis from different bacterial isolates. The results showed that well-dispersed nanoparticles and homogenous in diameter and shape.[13]

X-ray diffraction (XRD examination)

XRD analysis provided a perfect indicator of high-quality crystalline AgNPs using two different bacteria as a biosynthesis procedure, the crystalline nature of the silver nanoparticles in this study agree with other study that using *Klebsiella pneumonia* to precipitate AgNPs has been analyzed with the XRD diffraction pattern[14]

Fourier transform infrared spectroscopy (FTIR analysis)

So, from previous observation, the presence of the hydroxyl groups and primary amines are the most likely functionalities that can contribute to the reduction of silver nitrate to AgNPs. It is a technique utilized to get data about chemical bonding in a material. The band locations and amounts of absorption peaks are dependent on crystalline structure, chemical composition, and also on morphology.[15] FTIR measurements were reinforced to identify the probable biomolecules that can be liable for capping leading to proficiency of the silver nanoparticles.[16] The refined suspension containing the nanoparticles was completely dried and ground with KBr pellets and studied.

Antimicrobial activity of AgNPs

Several studies have demonstrated the efficacy of AgNPs as antimicrobial agents, and in some cases, they have been found to be more effective than conventional antibiotics against certain bacterial strains.[17] It is found that AgNPs exhibited rapid killing activity against all tested Gram-negative bacteria, reducing the bacterial count by over 99.9% within 1–2 h.[18] This suggests that AgNPs might be developed as a new type of antimicrobial agent, especially for treating multidrug-resistant bacterial infections.[19] It is reported that AgNPs showed much stronger antimicrobial activity against a broad spectrum of both Gram-negative and Gram-positive bacterial strains compared to the plant extracts used for their synthesis[20] confirmed that biogenically synthesized AgNPs exhibited a more significant antimicrobial effect while maintaining low cytotoxicity compared to chemically produced AgNPs. Gurunathan[21] observed that combinations of antibiotics and AgNPs showed significant antimicrobial effects even at sub-lethal concentrations of the antibiotics.

Free radical scavenging activity assay

Furthermore, the quinoid compound formed by oxidation of the phenol group in phenols may be adsorbed on the surface of Nanoparticles, providing suspension stabilization.[22,23] It is well known that phenolic compounds can participate directly in anti-oxidative action with other ingredients because the phenols reported have redox properties that enable them to act as a reducing factor, hydrogen donors, and singlet oxygen quenchers.[24] The same finding of scavenging activity of AgNPs was mentioned by many authors:[25] revealed the anti-oxidative effect mechanism of the AgNPs is proposed to be due to the scavenging of free radicals produced in the oxidation[26-29] suggesting that AgNPs have a minor inhibitory effect on Scenedesmus obliquus that leads to changes in 30 metabolites, including carbohydrates, and glycine, which increases to counteract intracellular oxidative stress.

Conclusion

Delftia acidovans (OM838393.1) strain can be used for the bio-synthesis of AgNPs in an environmentally friendly manner. These nanoparticles work well as antibacterials and antioxidants. It may also be synthesized on a large scale, and used to target resistant infectious bacteria it is strongly advised that it be used as a more affordable alternative to conventional anti-bacterial agents; or used as a drug delivery system to improve vascular dysfunction brought on by diabetes, hypertension, or atherosclerosis.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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