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Detection of anti-bacterial activity of silver nanoparticles from actinomycetes and testing its efficacy

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ABSTRACT

Silver nanoparticles rank among the most promising metal nanoparticles that are less than 100 nm in diameter. Their compact dimensions and substantial surface area-to-volume ratio lend them distinct physical, chemical, and biological properties that make them appealing for a multitude of applications. They can be synthesized in a variety of physical, chemical, and biological methods, and one such technique is the green route synthesis of AgNPs from actinomycetes. While AgNPs have immense potential in a diverse range of applications, its bactericidal effect is the focus of this current work. AgNPs' antibacterial activity was evaluated in both gram-positive and gram-negative bacteria at concentrations ranging from 25 to 150 μg. The bactericidal effect was more pronounced in gram-negative bacteria when compared to gram-positive bacteria. They exhibit twin actions of bactericidal assault and membrane rupture, which enable them to induce cell lysis and interfere with bacterial protein synthesis. Often alluded to as "Nano-antibiotics," these compounds may also serve as delivery systems for conventional antibiotics.

1. Introduction

Nanoparticles serve as the elemental building blocks for various nanotechnology applications. It plays an ever-expanding part in science, research, and advancement, as well as in everyday life. The history of nanomaterials is very long and major improvements within nanoscience have taken place in the last two decades. The idea of nanotechnology was first highlighted by Nobel laureate Richard Feynman, in his popular address at the California Institute of Technology on 29th December, 1959. One of his articles, published in 1960 and titled "There is Plenty of Room at the Bottom," discussed the idea of nanomaterials. Depending upon the shape and size of colloidal metal particles play pivotal parts in different sectors, including the preparation of magnetic and electronic gadgets, wound recuperating products, antimicrobial, gene expression, and preparation of biocomposites and noble metal colloids have the optical, catalytical, and electromagnetic properties.[1] It has diminished estimate with expanded greatness range of grip, stabilization, and controlled flocculation of colloidal dispersion.

2. Classification of Nanoparticles

Nanoparticles fall into two categories: mainly organic and inorganic nanoparticles. Organic nanoparticles include carbon nanoparticles (fullerenes) in other hand, inorganic nanoparticles may include magnetic nanoparticles, noble metal nanoparticles (gold and silver) and semi-conductor nanoparticles (like titanium dioxide and zinc oxide).

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2.1. Silver Nanoparticles

Silver nanoparticles are an intriguing class of nanomaterials that have gained an abundance of scrutiny recently due to their unique characteristics, which make them suitable for tackling an assortment of technological and therapeutic challenges. The features of this transition metal encompass optical, electrical, mechanical, catalytic, antimicrobial and antioxidant properties.[1,2] It is also used in food packaging, health additives, cosmetics, medication delivery, insecticides and bio-sensing. Inorganic nanoparticles are becoming more and more prominent because they offer superior material qualities and functional diversity.

Strong bactericidal activity of silver has been found against gram-positive and gram-negative bacteria, including strains that are resistant to many antibiotics. They have also been researched as possible tools for medical imaging and disease management; because of their large surface area and size properties, they have remarkable reactivity and functional benefits over chemical imaging agents and medicines.

The development of reliable methods for the generation of silver nanoparticles is a top focus in current nanotechnology research. They can be produced using a variety of techniques, while green route synthesis being one such feasible technique.[3–5] Silver is a health additive in traditional Chinese and Indian Ayurvedic medicine. The trend towards miniaturization and the necessity for modernization have led to a large surge in scientific publications devoted to the production and analysis of silver nanoparticles.

3. Objective of the Study (Methodology)

1. Biosynthesis of silver nanoparticles
2. Characterization of silver nanoparticles
3. Testing of anti-bacterial efficiency

4. Green Route Biosynthesis of Silver Nanoparticles[6–8]

4.1. Intracellular synthesis method

By adding silver nitrate, a solution of heavy metal ions was added to bacterial biomass for the intracellular production of silver nanoparticles was achieved. Bacteria were maintained alive on actinomycetes isolation agar. A prepared and sterilized 250 ml conical flask containing approximately 100 ml of nutrient broth, a solution of 1mM silver nitrate was added to the isolated culture and then incubated at room temperature in a rotary shaker at 120 rpm.

4.2. Extracellular synthesis method

The supernatant culture of actinomycetes was used in the process of extracellular synthesis. It was grown in 100 ml of sterile nutrient broth at 28°C for 24-48 hours in a rotary shaker at 220 rpm. After incubation, the culture was centrifuged at 10,000 rpm for 10 minutes and the supernatant was collected. 1mM of silver nitrate was added to this supernatant and the color change was noted after incubation. The color change indicates the formation of silver nanoparticles (Figure 1) and is periodically analyzed by a UV-vis spectrophotometer at different wavelengths.

Figure 1: Formation of silver nanoparticles by intra and extracellular method

5. Characterization of Silver Nanoparticles

5.1. UV-vis spectrophotometer analysis

This is a very useful technique to characterize the synthesized silver nanoparticles. UV-vis spectroscopy is used to study nanoparticles with controlled size and shape.

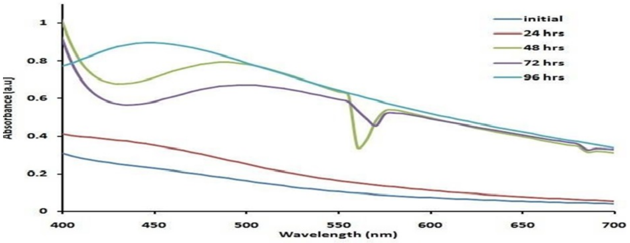


Figure 2: UV-vis spectra of intracellular reaction mixture using actinomycetes

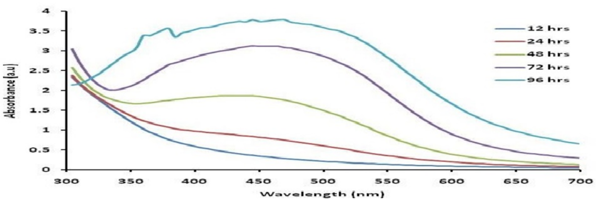


Figure 3: UV-vis spectra of extracellular synthesis of silver nanoparticles

The reduction of silver ions was monitored in the reaction medium in the wavelength range of 300-700 nm using a double beam UV-vis spectrophotometer (Perkin Elmer, Singapore) equipped with a 1000 mm quartz cell with a resolution of 1 nm and the spectrum of intra and extracellular reaction is as follows (Figures 2 and 3), at the different functional time with its wavelength on the x-axis and its absorbance on the y-axis.

5.2. Scanning electron microscope (SEM)

The morphology and size of silver nanoparticles were determined by using a scanning electron microscope (Philip model CM 200). A thin film of the sample was prepared on a carbon-coated copper grid by simply dropping a very small amount of sample and excess was removed with blotting paper, the grid was dried under a mercury lamp for 5 minutes and the image was recorded (Figure 4).[5]

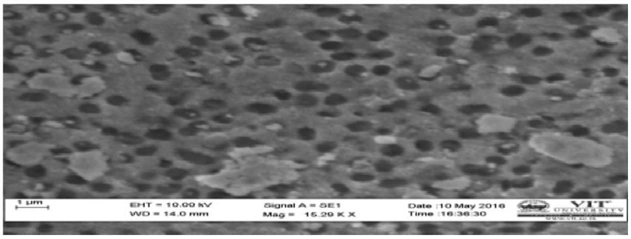


Figure 4: SEM image of AgNPs at the magnification of 1μm

5.3. Energy dispersive X-ray analysis (EDAX)

Elemental analysis of silver was performed by EDAX (Philips XL 30). Aqueous suspensions of silver nanoparticle samples were prepared for analysis using a drop-coating method. It was performed in spot profile mode with a beam diameter of 1μm at several locations on the sample.[9] Energy-dispersive X-ray spectrometry analysis confirmed the presence of elemental silver in the solution (Figure 5).

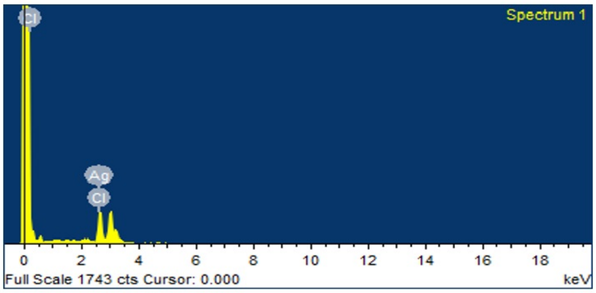


Figure 5: EDAX pattern of silver nanoparticles

6. Testing of Anti-Bacterial Efficiency[10–12]

This assay was conducted using the agar well diffusion method. Spreading was done using test organisms, *i.e.,* two gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and three gram-negative organisms (*Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa*). The culture was done on sterile muller hinton agar plates separately. The suspension was used to inoculate at 90 mm diameter. Wells were punched in triangular form and filled with silver nanoparticles at different concentrations from 25-150 μg/ml into each well on all plates. Distilled water was taken as negative control and Chloramphenicol was taken as positive control. Leave all the plates overnight at 37°C in the incubator. Following incubation, the anti-bacterial action of silver nanoparticles showed an inhibition zone, but bacteria showed full growth in distilled water. Results were observed (Figure 6), and the size of the zone of inhibition of silver nanoparticles against the tested organisms was measured, and the graph was plotted by the concentration of AgNPs on the x-axis and the zone of inhibition on the y-axis (Figure 7).

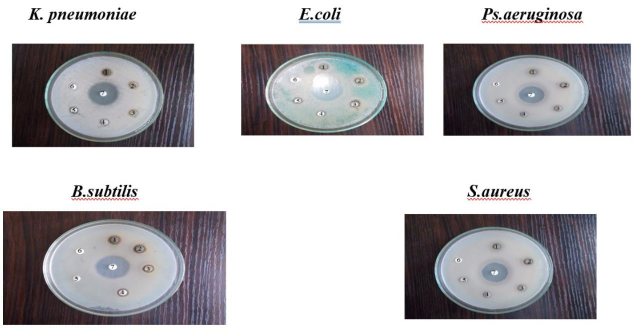


Figure 6: Plates showing the zone of inhibition of silver nanoparticles

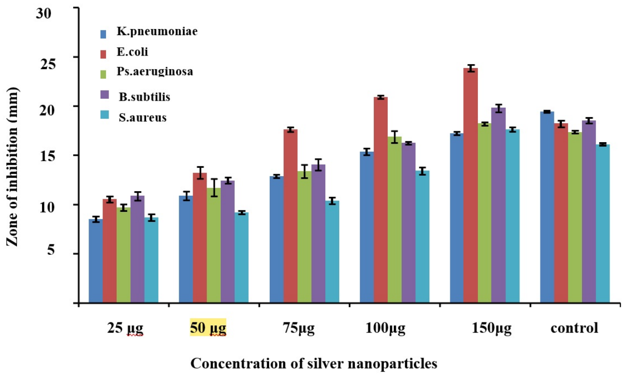
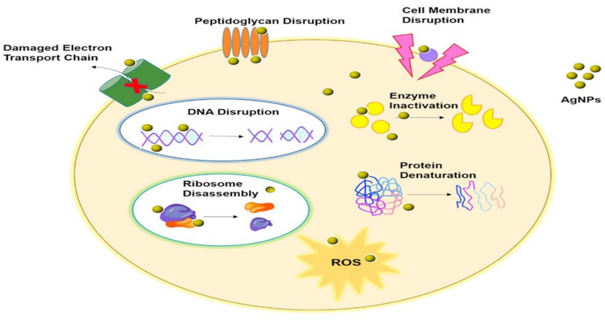


Figure 7: The anti-bacterial efficacy of silver nanoparticles against tested organisms

7. Mechanism of Anti-Bacterial Activity

1. The chemical makeup of gram-positive and gram-negative bacterial cell walls varies. Gram negative bacteria exhibit a non-rigid cell wall composed of a lipopolysaccharide bilayer on the exterior and a thin layer of peptidoglycan (7-8 nm) underneath. This bilayer was made conceivable by a covalent link between the lipid and polysaccharide. Metallic nanoparticles that are weak and positively charged become attracted to lipopolysaccharides owing to their negative charge.[13]
2. The main structural component of gram-positive bacteria is a thick layer (20-40 nm) of peptidoglycan, which is made up of short peptide chains that are cross-linked to generate a three-dimensional rigid structure. The rigid and extended cross-linking not only endows the cell wall with fewer anchoring sites for the AgNPs but also makes it difficult to penetrate. So that nanoparticles penetration was much more in gram-negative bacteria than the gram-positive bacteria.[14] Thus, the nanoparticles of silver are more toxic to gram-negative than gram-positive bacteria.
3. The mechanism of anti-bacterial activity of silver nanoparticles can be possible in several ways. Bacteria are having the strong negative charge, this charge may contribute to the sequestration of free Ag^{+} ions by electrostatic attraction. Bactericidal effects of silver nanoparticles obeyed a dual action mechanism of antibacterial activity, i.e., the bactericidal effect and membrane disrupting effect of the polymer subunits.[15]
4. Silver nanoparticles can attach to the surface of the cell membrane and disturb its power functions such as permeability, respiration and replication. Due to the generation of the Reactive Oxygen Species (ROS). It is reasonable to state that the binding of the particles to the bacteria depends on the surface area available for interaction. Smaller particles having the larger surface area available for interaction will give more bactericidal effect than the larger particles.[16] Silver nanoparticles were bind with bacterial DNA and affect the protein synthesis. Hence it results in cell lysis/death of the bacterium.



8. Applications of Silver Nanoparticles[8–10,17,18]

1. *Diagnostics:* Silver nanoparticles are used in biosensors and many test assays and it can be used as biological markers for quantitative detection.
2. *Antimicrobial coatings:* Silver nanoparticles are increasingly used in antimicrobial coatings for clothing, shoes, paints, wound dressings, instruments, cosmetics, polymers and much more products.
3. *Conductive material:* To improve thermal and electrical conductivity, silver nanoparticles are incorporated into composite materials and used in conductive inks for printing electronic circuits.
4. *Optical devices:* Collect light more effectively with Surface-Enhanced Raman Scattering (SERS) and Metal-Enhanced Fluorescence (MEF).
5. *Textile industry:* Nano silver colloid by impregnation method to ensure whiteness, silver content and wash resistance of silver treated fabrics.
6. *Biomedical devices:* Contains silver nanoparticles that continuously release small amounts of silver ions to protect the instrument against microorganisms.
7. *Computers and electronics:* Primarily used for its high electrical and thermal conductivity, chemical stability and ability to conduct electricity in the form of oxides. Nanoelectronics uses nanotechnology in electronic components such as flash memory chips, keyboards, cell phone castings, refrigerators,etc..
8. *Personal care products:* Nanosilver is used in soaps, toothpastes, pipes, deodorants, lip products, facial and body clays.
9. *Cosmetic Industry:* The cosmetic industry uses a variety of nanomaterials such as liposomes, ethosomes, solid lipid nanoparticles, nanocapsules, cubosomes and nanoemulsions. Cosmetics incorporating nanoscience are now widely available on the market.
10. *Antimicrobial agents:* AgNPs has highly antimicrobial property to several species of bacteria, nano-sized silver have the capability to provide a more durable antimicrobial protection, often for the life of the product.

9. Results and Discussion

Actinomycetes-mediated synthesis of silver nanoparticles was achieved and it has the ability to degrade silver ions into silver nanoparticles. The Surface Plasmon Resonance (SPR) band was observed at 450 and 460nm for both intra and extracellular synthesis method by UV-vis Spectrophotometry analysis. It depends on various factors such as the dielectrically constant of the medium, excitation vibrations, size, shape and stability of the particles, type of capping agent as well as refractive index of the surrounding medium. SEM images show highly aggregated and irregular spherical shapes with high cohesiveness. The size of nanoparticles is 80-90 nm at magnification of 1μm. Agglomeration occurs because there is not enough protective and capping agent in the culture supernatant. The presence of an excessive amount of reducing moieties bound to the particle surface may be responsible for the strong aggregation.

EDAX identification lines for the major emission energies for silver (Ag) are displayed at 3 keV, indicating the presence of silver nanoparticles. Some of the weak peaks, like chloride (Cl-), were also observed during examination. These are formed due to the X-ray emission from carbohydrates/proteins/enzymes present within the isolates. On MHA plates showed a clear inhibition zone at various concentrations against tested organisms, the inhibitory zone was raised while increasing the concentration of silver nanoparticles were investigated and compared their bactericidal activity.

10. Conclusion

This study shows that the green route synthesized silver nanoparticles have the potential to act against bacteria effectively. Predominantly against gram-negative bacteria than gram-positive bacteria, due to their cell wall chemistry. Its efficacy arises from the ability of AgNPs to interact with their cell membranes, disrupt cellular processes and induce oxidative stress, ultimately leading to the microbial death. Among tested organisms it showed significant anti-bacterial action against *Escherichia coli* with low concentration of silver nanoparticles. The maximum inhibition zone was obtained at the concentration of 150 μg. They have the ability to kill microbes effectively by both intra and extracellularly. Silver nanoparticles have a significant role in nanoscience and nanomedicines to treat and prevent various diseases in humans. Current research in inorganic nanomaterials having good antimicrobial properties has opened a new era in the medical and pharmaceutical industries.

11. Source of Funding

None.

12. Conflict of Interest

None.

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