

Biogenesis of silver nanoparticles using the bacteria and its antibacterial study

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Abstract

The environmentally friendly nanoparticles are created using a technology that uses microbes like bacteria as an alternative to the traditional chemical process. The silver nanoparticles in the current study were created using gram-negative *E. coli* that was isolated from contaminated soil (AgNPs). After being incubated for 3 to 5 days at room temperature, the bacteria's enzyme reduced the silver nitrate solution to create the AgNPs. UV-Vis spectroscopy later confirmed a color shift from pale white to brown as an early sign of the synthesis. FTIR and SEM measurements were used to further characterize the AgNPs. It was discovered that the produced nanoparticles were spherical and had a small amount of aggregation. Their size ranged from 20 to 40 nm. The presence of elemental silver was confirmed by the energy-dispersive spectra of the nanoparticle dispersion. It was discovered that the AgNPs had antibacterial properties. Silver nanoparticles with potential antibacterial properties were produced by the *E. coli* bacteria.

Keywords: AgNPs, *E. coli*, Green synthesis, Antibacterial activity.

1. Introduction

Due to its numerous applications, research in the field of nanotechnology has recently received significant attention. Because of this, the physical, chemical, biological, and engineering sciences were combined to create novel manufacturing and control methods for nanomaterials, which are gaining attention due to their more pronounced properties at such small sizes. When compared to the microlevel, certain phenomena may be more clearly displayed at the nanoscale. The material's mechanical, thermal, and catalytic properties are altered by the material's increased surface area to volume ratio, one important property. the atomic level manipulation of the material. As the ratio of surface area to volume increases, the behaviour of atoms on the surface of the particle takes precedence over that of atoms within, altering the properties of the particle. Inorganic nanoparticles are becoming increasingly important as potential tools for medical imaging and disease treatment due to their advantages in size over chemical imaging agents and drugs. Inorganic nanomaterials have been extensively utilized for cellular delivery because of their adaptability [1]. These characteristics include their capacity for controlled drug release and targeted drug delivery, rich functionality, good biocompatibility, and wide availability.

Traditionally, only physical and chemical processes were used to create nanoparticles. However, these methods use harmful chemicals in the synthesis process, which raises environmental concerns such as the production of hazardous byproducts, the use of toxic solvents, and contamination from precursor chemicals[2]. In some of these methods, nanoparticle aggregation, controlled crystal growth, and stability are also issues. Consequently, a brand-new field of study has emerged: the production of various nanoparticles through the utilization of biological systems like microbes. The successful synthesis of nanoparticles with potential antibacterial activity was demonstrated by numerous studies on the microbial synthesis of nanoparticles[3-8].

One of these groups is the endophytes, a group of microbes that have the ability to biogenically produce nanoparticles. Endophytes, according to Bacon and colleagues, are "microbes that colonize living internal tissues of plants without causing any immediate, overt negative effects"[9]. Strobel and colleagues, on the other hand, suggested that the relationship might be mutualistic or borderline pathogenic. The majority of endophytes coexist with bacteria and fungi [11]. Nanoparticles have only been made with endophytic fungi in a few reports. *Colletotrichum* species An endophytic fungus was used in one of these studies. isolated from the geranium (*Pelargonium graveolens*) leaves for use in the extracellular synthesis of gold nanoparticles [12]. According to

another study[13], the endophytic fungus *Aspergillus clavatus* (AzS-275), which was isolated from sterilized stem tissues of *Azadirachta indica*, produced silver nanoparticles.

Nanoparticle synthesis by endophytic bacteria to date. Silver nanoparticles could be made by utilizing the endophytic bacterium of a medicinal plant. The synthesized nanoparticles' antibacterial activity has been evaluated and characterized.

2. Materials and methods

- a. Isolation of bacteria: *E. coli* and *Streptococcus aureus* (*S. aureus*) were isolated from contaminated water using MFC7 Hr Agar Media. And After isolation, pure culture of *S. Aureus* was spread over the Nutrient Agar Media for further antibacterial studies using well diffusion method.
- b. Synthesis of silver nanoparticles: *E. coli* bacteria cultures were inoculated into 100 milliliters of media. One of these groups is the endophytes, a group of microbes that have the ability to biogenically produce nanoparticles. Endophytes, according to Bacon and colleagues, are "microbes that colonize living internal tissues of plants without causing any immediate, overt negative effects"[9]. Strobel and colleagues, on the other hand, suggested that the relationship might be mutualistic or borderline pathogenic. The majority of endophytes coexist with bacteria and fungi [11]. Nanoparticles have only been made with endophytic fungi in a few reports. *Colletotrichum* species An endophytic fungus was used in one of these studies. isolated from the geranium (*Pelargonium graveolens*) leaves for use in the extracellular synthesis of gold nanoparticles [12]. According to another study[13], the endophytic fungus *Aspergillus clavatus* (AzS-275), which was isolated from sterilized stem tissues of *Azadirachta indica*, produced silver nanoparticles.

c. Characterization of nanoparticles

X-Ray Diffraction Analysis (XRD):

Using CuK α as the radiation source, an X-ray diffractometer at a wavelength of 1.5406 Å confirmed the synthesis of Ag-NPs. Silver oxide's crystalline structure and phase were examined using the XRD method in the 2 θ range of 30–70.

Fourier Transform Infra-Red Microscopy (FTIR):

This method is used to determine the functional groups that are present in synthesized copper oxide NPs because each chemical bond has an energy absorption band that is used to examine the structural and bond information of a complex in order to study the type of bonding and its strength. The synthesized samples' FTIR spectra, which ranged from 4000 to 400 cm⁻¹ with a resolution of 4 cm⁻¹, were obtained using the KBr pellet method.

Antibacterial Activity:

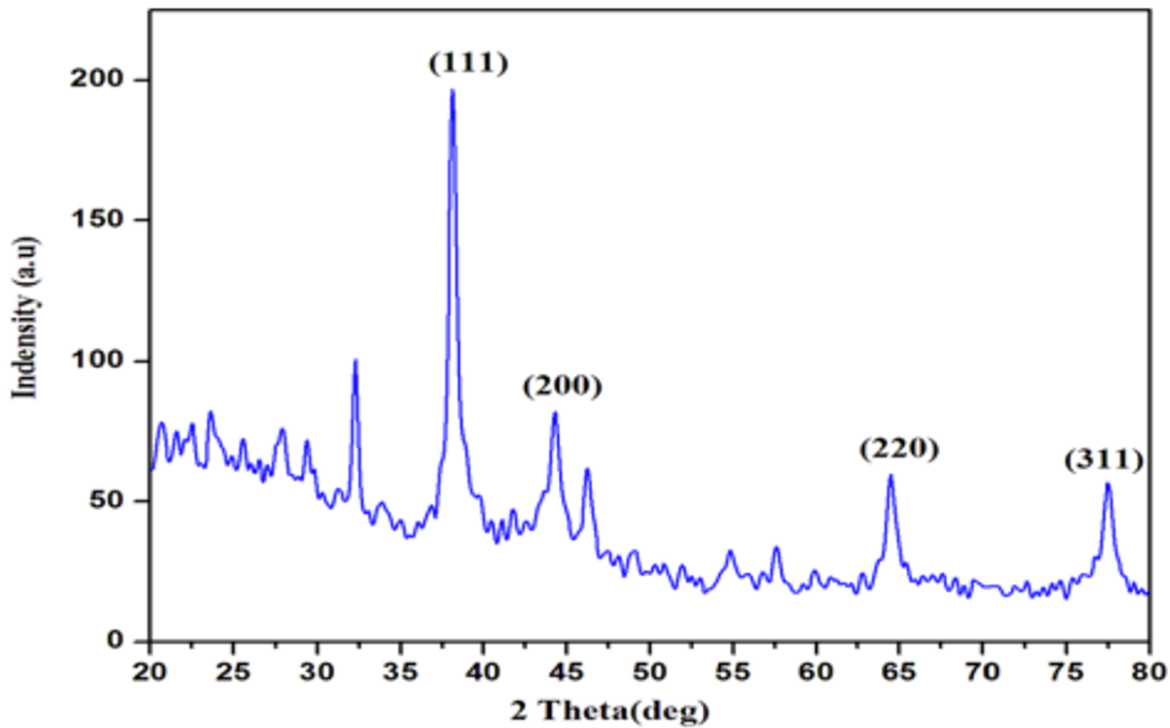
After the media had solidified, cotton swabs were used to swab the surface of the NAM media with fresh bacteria cultures that had been adjusted to the standard 0.5 McFarland turbidity. Using a sterile cork borer, all Petri plates in the agar well diffusion method had three wells (6 mm in diameter). As a negative control, silver oxide nanoparticles and the solvent DMSO are present in each plate well. The measurement of inhibition zones followed [26].

3. Results and Discussion

X-Ray Diffraction Analysis. The observed peaks confirm that bacteria were used in the green method to produce silver oxide nanoparticles. The peak position is at 2 θ of 32 and 63.6, which were assigned to the planes (110), (111), (111), (200), (202), (002), (113), (220), and (312), which are consistent with the Ag-NPs from JCPDS card 801916 in a good way. The formation of crystalline monoclinic morphology is supported by this. The crystalline nature of Ag nanoparticles is confirmed by the sharp, clearly defined reflections of silver oxide nanoparticles on XRD patterns [29]. In addition, the crystallite size of Ag-NPs was determined using the Debye-Scherrer equation.

$$D = \frac{k\lambda}{\beta \cos \theta}$$

where k is the Scherrer constant, θ is the Bragg angle, β is the peak broadening half the maximum intensity, and λ is the X-ray wavelength (1.5418 Å), respectively. Consequently, the XRD analysis revealed that the particles initially engage in collisions before tending to expand and further react with the surrounding O₂.



Fourier Transform Infra-Red Microscopy (FTIR): The 400–4000 cm^{-1} FTIR spectrum of silver oxide nanoparticles at room temperature is shown in Figure 2. Bands at 3340.21 cm^{-1} , 1636.58 cm^{-1} , 1562.35 cm^{-1} , 1412.25 cm^{-1} , 1021.14 cm^{-1} , 800.58 cm^{-1} , 600.14 cm^{-1} , and 518.39 cm^{-1} can be found in the Ag-NPs' infrared (IR) spectrum. Between 518.4 and 1021.1, the typical silver oxide peaks are found 1021.1 cm^{-1} .

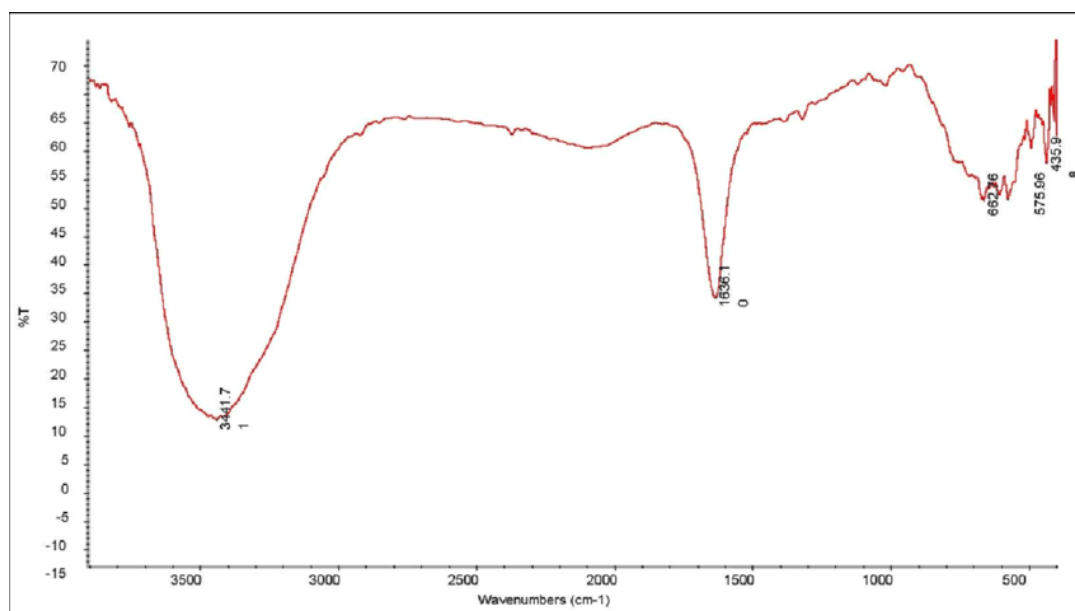


Figure 2:FTIR spectrum of AgNPs

1636.1 cm¹ represent O–H bending and C–C stretching, respectively. N–H stretching is attributed to the peak at 3440.2, which could be caused by an amino acid that also serves as a capping agent [27].

Antibacterial Activity of silver Oxide Nanoparticles At a concentration of 50 g/mL, Ag-NPs were tested for their ability to kill *S. aureus*. DMSO was used as a negative control, and Norfloxacin was used as a positive control. Ag-NPs were very effective against *S. aureus*, as evidenced by the sample's 9 1 mm maximum inhibition zone.

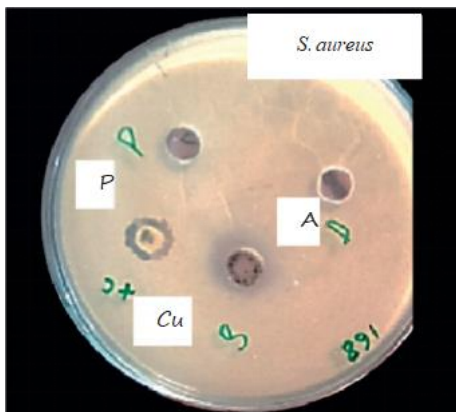


FIGURE4: Antibacterial activity of Ag-NPs against S. aureus.
TABLE1: Different inhibition zone of Ag-NPs against selected bacterial strains.

| | <i>E. coli</i> | <i>P. aeruginosa</i> | <i>S. aureus</i> | <i>Acinetobacter</i> |
|-------------|----------------|----------------------|------------------|----------------------|
| Ag-NPs | 5 ± 1 | 5 ± 1 | 9 ± 1 | 5 ± 1 |
| Norfloracin | 12 ± 0 | 5 ± 0 | 4 ± 0 | 3 ± 1 |
| DMSO | 0 | 0 | 0 | 0 |

Norfloracin as a positive control; DMSO dimethyl sulfoxide as negative control; ± standard error mean.

Discussion

Due to their significance as catalyst, ceramic resistor, superconducting material, gas sensor, and roles in the pharmaceutical and energy industries, silver oxide nanoparticles have piqued the interest of numerous researchers [36]. The current study focused on how to make silver oxide nanoparticles using E. coli bacterial extract. Silver oxide nanoparticles' crystal structure and phase were discovered through X-ray diffraction analysis. The findings from XRD demonstrated that the particle size was 15 nm. Silver oxide nanoparticles' various functional groups and biomolecules were identified using FTIR spectroscopy. Different peaks in the result suggested the development of Ag nanostructures

4. Conclusion

The green synthesis of silver oxide nanoparticles using E. coli bacterial extract is found to be simple, cost-effective, and non-toxic. XRD analysis of the size of the Ag nanoparticles, which was around 15 nm, confirmed their crystalline structure. FTIR confirmed the macromolecules' physical interactions with Ag-NPs and various function groups. The cytotoxic, antibacterial, and antifungal properties of the Ag nanoparticles were desirable. The investigation into the toxicity of synthesized nanoparticles revealed that, when used in industry at concentrations below 60 g/mL, these nanoparticles typically pose little risk.

Data Availability

All the available data are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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