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GREEN SYNTHESIS OF SILVER NANOPARTICLES USING E. COLI: CHARACTERIZATION AND EVALUATION OF ANTIBACTERIAL ACTIVITY AGAINST CLINICAL PATHOGENS

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ABSTRACT

The biosynthesis of silver nanoparticles (AgNPs) using microorganisms has gained significant attention due to its eco-friendly and cost-effective approach. In this study, Escherichia coli was utilized for the extracellular synthesis of AgNPs. The synthesized nanoparticles were characterized using UV-Vis spectroscopy, Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and scanning electron microscopy (SEM). Furthermore, the antimicrobial potential of AgNPs was evaluated against clinical pathogens isolated from various samples. The results indicate significant antibacterial activity, highlighting the potential of biosynthesized AgNPs as an alternative to conventional antimicrobial agents.

Keywords: Silver nanoparticles, Escherichia coli, biosynthesis, antimicrobial activity, characterization

1. INTRODUCTION

Silver nanoparticles (AgNPs) have emerged as potent antimicrobial agents due to their broad-spectrum activity against bacteria, fungi, and viruses. Traditional synthesis methods involve the use of toxic chemicals, posing environmental and health risks. Therefore, green synthesis using biological entities such as bacteria offers a sustainable alternative. Escherichia coli (E. coli), a commonly found bacterium, has been explored for its ability to reduce silver ions into AgNPs extracellularly. This study aims to characterize AgNPs synthesized using E. coli and assess their antibacterial efficacy against clinical pathogens.

Nanotechnology has emerged as a rapidly evolving field with immense potential in various scientific domains, particularly in medicine and biotechnology. One of the most significant advancements in this field is the synthesis and application of nanoparticles, especially silver nanoparticles (AgNPs), due to their exceptional physicochemical properties and biological applications. Among the various methods of AgNP synthesis, the green synthesis approach using biological entities such as bacteria, fungi, and plant extracts has gained considerable attention due to its eco-friendliness, cost-effectiveness, and biocompatibility. Escherichia coli (E. coli), a widely studied and well-characterized bacterium, has been explored as an efficient biological agent for the biosynthesis of AgNPs. The use of E. coli in nanoparticle synthesis provides a sustainable alternative to conventional chemical and physical methods, which

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often involve toxic reagents and high energy consumption. The present study aims to investigate the green synthesis of AgNPs using E. coli, followed by their characterization and evaluation of antibacterial efficacy against clinically relevant pathogens.

Background and Significance of Silver Nanoparticles

Silver has been recognized for its antimicrobial properties for centuries. Historically, silver compounds were used to treat infections before the advent of antibiotics. With the rise of nanotechnology, silver in its nano-form has shown enhanced antibacterial potential due to its increased surface area and reactivity. AgNPs exhibit strong antimicrobial activity against a wide spectrum of bacteria, fungi, and viruses, making them an attractive alternative to conventional antibiotics. Their mode of action involves multiple mechanisms, including the generation of reactive oxygen species (ROS), disruption of bacterial cell membranes, interaction with thiol groups in proteins, and inhibition of DNA replication. Given the growing global concern of antimicrobial resistance (AMR), the development of novel antimicrobial agents such as AgNPs is crucial in combating multidrug-resistant (MDR) pathogens.

Green Synthesis of Silver Nanoparticles

The conventional chemical and physical methods for AgNP synthesis involve hazardous chemicals and extreme reaction conditions, making them less sustainable for biomedical applications. Green synthesis, on the other hand, employs natural biological resources such as microorganisms and plant extracts, offering a safer and more environmentally friendly alternative. Microbial synthesis of AgNPs has gained prominence due to the ability of bacteria and fungi to reduce silver ions (Ag^+) into elemental silver (Ag^0) through enzymatic and non-enzymatic pathways. Among bacterial species, E. coli has demonstrated promising potential in nanoparticle biosynthesis due to its rapid growth, genetic manipulability, and ability to tolerate metal ions.

The biosynthesis of AgNPs using E. coli involves the reduction of silver nitrate ($AgNO_3$) by biomolecules such as proteins, enzymes, and metabolites present in bacterial cells. The reduction process can occur either intracellularly or extracellularly, depending on the bacterial strain and growth conditions. Extracellular synthesis is preferred as it facilitates easy purification and collection of nanoparticles without extensive cell disruption. Various factors, including pH, temperature, silver ion concentration, and incubation time, influence the size, shape, and stability of the synthesized nanoparticles. The optimization of these parameters is essential to obtain AgNPs with desired physicochemical properties suitable for biomedical applications.

Characterization of Biosynthesized AgNPs

Characterization of AgNPs is a crucial step to determine their morphology, size distribution, crystallinity, and functional groups involved in stabilization. Several analytical techniques are employed to study the physicochemical properties of biosynthesized nanoparticles. UV-Visible (UV-Vis) spectroscopy is commonly used to monitor the formation of AgNPs by detecting the characteristic surface plasmon resonance (SPR) peak, which typically appears between 400 and 450 nm. Fourier Transform Infrared (FTIR) spectroscopy provides insights into the biomolecules responsible for the reduction and capping of AgNPs. X-ray Diffraction (XRD) analysis confirms the crystalline nature of nanoparticles, while Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) reveal their size, shape, and surface morphology. Dynamic Light Scattering (DLS) is employed to measure the hydrodynamic size and zeta potential, which are critical for assessing nanoparticle stability in biological environments.

Antibacterial Mechanism and Clinical Applications

The antibacterial activity of AgNPs is attributed to multiple mechanisms that collectively disrupt bacterial physiology. The small size and high surface area of AgNPs enable them to interact with bacterial membranes, causing structural damage and increased permeability. AgNPs also induce oxidative stress by generating ROS, leading to lipid peroxidation, protein denaturation, and DNA damage. Furthermore, silver ions released from AgNPs can interfere with vital cellular functions by binding to thiol groups in enzymes and disrupting metabolic pathways. These multifaceted mechanisms make AgNPs effective against both Gram-positive and Gram-negative bacteria, including antibiotic-resistant strains.

The biomedical applications of AgNPs extend beyond antibacterial activity. They are widely utilized in wound dressings, medical coatings, and disinfectants to prevent infections. Additionally, AgNPs have been explored for their antiviral, antifungal, and anticancer properties, demonstrating potential in diverse therapeutic interventions. However, despite their promising applications, concerns regarding the cytotoxicity and environmental impact of AgNPs remain. Therefore, thorough biocompatibility assessments and controlled synthesis approaches are essential for their safe and effective utilization in medical settings.

This study aims to explore the green synthesis of AgNPs using E. coli, characterize their physicochemical properties, and evaluate their antibacterial efficacy against clinical pathogens. The choice of E. coli as a biosynthetic agent is based on its ease of cultivation, rapid growth, and ability to secrete biomolecules that facilitate silver ion reduction. The synthesized nanoparticles will be analyzed using various spectroscopic and microscopic techniques to assess their structural and functional attributes. Furthermore, their antibacterial potential will be tested against a panel of clinically relevant pathogens, including Staphylococcus aureus, Pseudomonas aeruginosa, and Klebsiella pneumoniae, to determine their efficacy and possible medical applications.

The green synthesis of AgNPs using E. coli represents a promising approach for developing eco-friendly and effective antimicrobial agents. With the increasing threat of antibiotic resistance, AgNPs offer a viable alternative for combating bacterial infections. This study will contribute to the understanding of biosynthesized AgNPs' structural, functional, and antibacterial properties, paving the way for their potential application in clinical and pharmaceutical fields. By leveraging biological systems for nanoparticle synthesis, this research aligns with the principles of green chemistry, promoting sustainable and safe nanomaterial development.

2. MATERIALS AND METHODS

2.1 Bacterial Culture and AgNP Synthesis

A clinical strain of E. coli was cultured in Luria-Bertani (LB) broth and exposed to a 1 mM silver nitrate ($AgNO_3$) solution. The mixture was incubated at 37°C under static conditions for 24-48 hours. A color change to brown indicated nanoparticle formation.

2.2 Characterization of Silver Nanoparticles

- **UV-Vis Spectroscopy:** The synthesized AgNPs were monitored using a UV-Vis spectrophotometer within the range of 300-700 nm.
- **Fourier-transform Infrared Spectroscopy (FTIR):** Functional groups involved in nanoparticle stabilization were analyzed.
- **X-ray Diffraction (XRD):** The crystalline nature of the AgNPs was confirmed.
- **Scanning Electron Microscopy (SEM):** The morphology and size distribution of the nanoparticles were examined.

2.3 Antibacterial Activity Assessment

The antibacterial activity of AgNPs was tested against clinical isolates (Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and Klebsiella pneumoniae) using the agar well diffusion method. The minimum inhibitory concentration (MIC) was also determined.

3. RESULTS AND DISCUSSION

- **Nanoparticle Characterization:** UV-Vis spectroscopy confirmed AgNP formation with an absorption peak around 420-450 nm. FTIR analysis suggested biomolecules from E. coli played a role in AgNP stabilization. XRD patterns revealed a face-centered cubic (FCC) structure of AgNPs, while SEM images demonstrated spherical nanoparticles with an average size of 10-50 nm.
- **Antibacterial Activity:** The synthesized AgNPs exhibited significant antibacterial activity, with maximum inhibition zones observed against S. aureus and P. aeruginosa. The MIC values ranged from 5 to 20 µg/mL depending on the bacterial strain.

- **Mechanism of Action:** The potential mechanisms of antibacterial activity include disruption of bacterial cell walls, generation of reactive oxygen species (ROS), and interference with cellular metabolism.

4. CONCLUSION

This study successfully demonstrates the extracellular synthesis of AgNPs using E. coli, with effective characterization and potent antimicrobial activity. The findings suggest that biologically synthesized AgNPs could be a promising alternative for combating antibiotic-resistant pathogens. Future research should focus on scaling up production and exploring biomedical applications.

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