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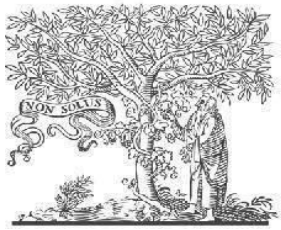

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EXTRACELLULAR BIOSYNTHESIS OF SILVER NANOPARTICLES BY ESCHERICHIA COLI: CHARACTERIZATION AND ANTIMICROBIAL POTENTIAL AGAINST CLINICALLY ISOLATED BACTERIA

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ABSTRACT

Silver nanoparticles (AgNPs) have gained immense attention due to their broad-spectrum antimicrobial activity and potential applications in medicine. This study investigates the extracellular biosynthesis of AgNPs using Escherichia coli, their characterization, and their antimicrobial efficacy against clinically isolated bacterial strains. The biosynthesis of AgNPs was confirmed through UV-visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR), dynamic light scattering (DLS), X-ray diffraction (XRD), and transmission electron microscopy (TEM). The antimicrobial potential was assessed against multidrug-resistant bacteria, demonstrating significant inhibition. These findings highlight the eco-friendly synthesis of AgNPs and their promising role as an antimicrobial agent.

Key words: biomedical applications, drug delivery, environmental impact, safety

1. INTRODUCTION

The emergence of multidrug-resistant (MDR) bacteria has necessitated the search for alternative antimicrobial agents. Silver nanoparticles (AgNPs) have exhibited strong antibacterial properties, making them a promising solution. Traditional chemical and physical methods for AgNP synthesis often involve toxic reagents, limiting their biomedical applications. Hence, biogenic synthesis, using microorganisms such as Escherichia coli, presents an environmentally sustainable alternative. This study aims to characterize biologically synthesized AgNPs and evaluate their antimicrobial effects against clinically relevant bacterial isolates.

Nanotechnology has emerged as a transformative field with applications across various scientific disciplines, including medicine, environmental science, and material engineering. Among the different types of nanoparticles, silver nanoparticles (AgNPs) have attracted significant attention due to their remarkable antimicrobial, antifungal, and antiviral properties. Silver has been used for centuries for its antimicrobial properties, but with advancements in nanotechnology, its efficacy has been enhanced manifold at the nanoscale. AgNPs can be synthesized using physical, chemical, and biological methods, but biological synthesis has gained popularity due to its eco-friendliness, cost-effectiveness, and sustainability. The biological approach utilizes microorganisms, plant extracts, or enzymes to

synthesize nanoparticles under mild conditions without the need for toxic chemicals or high-energy inputs. Among microorganisms, Escherichia coli, a widely studied and well-characterized bacterium, has been reported to mediate the extracellular biosynthesis of AgNPs efficiently. The extracellular biosynthesis of silver nanoparticles using E. coli involves the reduction of silver ions (Ag^{+}) to metallic silver (Ag^{0}) through biomolecules secreted by the bacterial cells. These biomolecules, including enzymes, proteins, and metabolites, act as reducing and stabilizing agents, ensuring controlled nanoparticle formation with desired physicochemical properties. One of the major advantages of extracellular synthesis is that it facilitates easy separation and purification of nanoparticles from the culture medium compared to intracellular synthesis, which requires cell lysis.

The biosynthesized AgNPs exhibit unique optical, electrical, and catalytic properties that make them highly useful in biomedical applications, including antimicrobial coatings, drug delivery, and wound healing. Characterization of the synthesized AgNPs is crucial to understanding their size, morphology, crystallinity, and stability, which significantly influence their biological activity. Various analytical techniques such as UV-visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), transmission electron microscopy (TEM), and dynamic light scattering (DLS) are commonly employed to analyze AgNPs. These techniques help determine the shape, size distribution, surface functional groups, and structural properties of the nanoparticles, ensuring their effectiveness for intended applications. The antimicrobial potential of biosynthesized AgNPs has been widely studied against both Gram-positive and Gram-negative bacteria, including clinically isolated multidrug-resistant pathogens.

AgNPs exhibit antimicrobial action through multiple mechanisms, including disruption of bacterial cell membranes, interference with protein synthesis, generation of reactive oxygen species (ROS), and inhibition of DNA replication, ultimately leading to cell death. Due to their nanoscale size, AgNPs can easily penetrate bacterial cells, making them highly effective even at low concentrations. The increasing prevalence of antibiotic-resistant bacteria has intensified the search for alternative antimicrobial agents, and AgNPs have emerged as a promising solution due to their broad-spectrum activity and reduced likelihood of resistance development. The green synthesis of AgNPs using E. coli not only provides an efficient and sustainable method for nanoparticle production but also aligns with the principles of green chemistry, minimizing environmental pollution.

However, challenges such as nanoparticle stability, cytotoxicity, and potential environmental risks need to be addressed before large-scale application. Ongoing research focuses on optimizing synthesis parameters, improving nanoparticle stability, and evaluating their biocompatibility for medical and industrial applications. the extracellular biosynthesis of silver nanoparticles using Escherichia coli offers an environmentally friendly, cost-effective, and efficient approach to nanoparticle production. Their potent antimicrobial properties, coupled with their unique physicochemical characteristics, make them highly valuable in various fields, particularly in combating drug-resistant bacterial infections. Further studies on

toxicity, stability, and large-scale production will be crucial for their successful integration into clinical and commercial applications.

2. MATERIALS AND METHODS

Biosynthesis of Silver Nanoparticles

E. coli was cultured in Luria-Bertani broth and incubated at 37°C. After reaching the exponential phase, silver nitrate (AgNO3) solution was added to the culture. The mixture was incubated under controlled conditions, leading to the extracellular reduction of Ag+ ions into AgNPs.

Characterization of Silver Nanoparticles

1. **UV-Vis Spectroscopy:** The formation of AgNPs was initially monitored using UV-visible spectroscopy in the range of 300-600 nm.
2. **Fourier-Transform Infrared Spectroscopy (FTIR):** FTIR analysis was performed to determine the functional groups involved in the bioreduction and stabilization of AgNPs.
3. **Dynamic Light Scattering (DLS):** DLS was used to analyze the size distribution and stability of AgNPs.
4. **X-ray Diffraction (XRD):** XRD confirmed the crystalline nature of the nanoparticles.
5. **Transmission Electron Microscopy (TEM):** TEM provided insight into the morphology and size of the biosynthesized AgNPs.

Antimicrobial Activity Assessment

The antimicrobial efficacy of the synthesized AgNPs was tested against MDR clinical isolates using the well-diffusion method and minimum inhibitory concentration (MIC) determination. The bacteria tested included Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Escherichia coli.

3. RESULTS AND DISCUSSION

The biosynthesis of AgNPs was confirmed by a characteristic UV-visible absorbance peak at approximately 420 nm. FTIR analysis identified proteins and biomolecules responsible for AgNP stabilization. TEM images revealed spherical nanoparticles with an average size of 10-50 nm. XRD patterns indicated face-centered cubic (FCC) crystalline structures, confirming the presence of silver. Antimicrobial assays demonstrated significant inhibition zones against MDR bacteria, with MIC values in the range of 2-10 µg/mL. The antimicrobial action is attributed to the disruption of bacterial membranes and interference with cellular metabolism.

4. CONCLUSION

The extracellular biosynthesis of AgNPs using E. coli presents an eco-friendly and cost-effective approach for nanoparticle synthesis. The characterized AgNPs exhibited strong antimicrobial activity against MDR bacterial strains, making them a potential candidate for biomedical applications. Further studies are needed to explore their mechanism of action and in vivo efficacy.

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