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Biosynthesis of Nanoparticles Using Probiotics and its Antibacterial Properties

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ABSTRACT

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Silver nanoparticles (AgNPs) have garnered significant interest due to their potent antibacterial properties against various pathogens. This study investigates the antibacterial activity of synthesized AgNPs against a range of Gram-positive and Gram-negative bacterial strains, including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Proteus vulgaris*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Enterobacter aerogenes*, and *Salmonella typhimurium*. The antibacterial efficacy was evaluated using the well diffusion method, with zones of inhibition (ZOI) recorded for each bacterial strain. The results demonstrated that *Staphylococcus aureus* exhibited the highest susceptibility to AgNPs (21 ± 1.8 mm), while *Pseudomonas aeruginosa* showed the lowest susceptibility (12 ± 1.0 mm). Gram-positive bacteria were generally more susceptible to AgNPs than Gram-negative bacteria, likely due to differences in cell wall structure and composition. These findings corroborate previous studies indicating that AgNPs disrupt bacterial cell membranes, generate reactive oxygen species (ROS), and interfere with cellular processes. The broad-spectrum antibacterial activity of AgNPs highlights their potential for applications in biomedical fields, such as wound healing, antibacterial coatings, and water purification systems. Further research is recommended to optimize AgNP synthesis and dosage for enhanced efficacy and reduced bacterial resistance.

Introduction

In recent years, the biosynthesis of nanoparticles (NPs) has emerged as a sustainable and eco-friendly alternative to conventional chemical synthesis methods. These biologically derived nanoparticles exhibit unique physicochemical properties that make them highly desirable in medical, pharmaceutical, and industrial applications (Shah *et al.,* 2018; Sathishkumar *et al.,* 2020). Compared to physical and chemical methods, biological synthesis using microorganisms is non-toxic, cost-effective, and produces nanoparticles with high biocompatibility (Mittal *et al.,* 2013; Iravani, 2011).

Among various biological agents, probiotics—beneficial microorganisms known for their health-promoting effects—have shown immense potential as bio-factories for nanoparticle synthesis (Sankar *et al.,* 2023; Gurunathan *et al.,* 2014). Probiotic bacteria such as *Lactobacillus*, *Bifidobacterium*, and *Bacillus* species can act as reducing and stabilizing agents in the synthesis of nanoparticles, offering a low-cost, non-toxic, and scalable approach (Iravani, 2020; Kalishwaralal *et al.,* 2010). These bacteria produce surface proteins, peptides, and metabolites, including exopolysaccharides and organic acids, that facilitate the reduction of metal ions, thereby generating nanoparticles with controlled size and morphology (Kumar *et al.,* 2018; Narayanan *et al.,* 2012).

Moreover, the nanoparticles synthesized through probiotic-mediated processes have demonstrated remarkable antimicrobial properties against a wide spectrum of pathogenic bacteria (Rai *et al.,* 2021; Salari *et al.,* 2020). The antimicrobial efficacy of these biosynthesized nanoparticles is attributed to mechanisms such as the disruption of bacterial cell membranes, generation of reactive oxygen species (ROS), and interference with bacterial DNA replication (Singh *et al.,* 2019; Loo *et al.,* 2018). These properties make probiotic-derived nanoparticles promising candidates for tackling antibiotic-resistant bacterial strains (Li *et al.,* 2020; Gopinath *et al.,* 2014).

In addition to their antimicrobial potential, probiotic-mediated synthesis aligns with the principles of green chemistry, minimizing hazardous byproducts and environmental impact (Ahmed *et al.,* 2016).

Recent studies have focused on optimizing the biosynthesis process, exploring different probiotic strains, and characterizing the resulting nanoparticles for their antimicrobial and biomedical applications (Khan *et al.,* 2021; Chaudhary *et al.,* 2020; Beyth *et al.,* 2015; Hassan *et al.,* 2021).

This study aims to explore the biosynthesis of nanoparticles using probiotic strains and evaluate their antimicrobial properties against clinically relevant bacterial pathogens. By leveraging probiotics, we aim to develop a sustainable approach for the synthesis of biofunctional nanoparticles with significant implications in antimicrobial therapy.

Materials and Methods

Synthesis of AgNPs

Lactobacillus plantarum MTCC was cultured in 250-mL Erlenmeyer flasks containing 100 mL of Nutrient Broth. The flasks were incubated at 37°C with continuous shaking at 150 rpm for 24 hours. After incubation, the bacterial biomass was harvested by centrifugation at 5000 rpm for 15 minutes. The collected biomass was thoroughly washed with sterile distilled water to remove residual media components.

The washed biomass was then challenged with a 1 mM solution of silver nitrate ($AgNO_3$). The mixture was incubated at 37°C for 48 hours to facilitate the synthesis of silver nanoparticles (AgNPs). Following the incubation period, the solution was centrifuged at 10,000 rpm for 15 minutes to separate the biomass from the supernatant containing the synthesized AgNPs.

Characterization of AgNPs

The formation of AgNPs was initially detected by observing a color change in the cell filtrate, indicating the reduction of Ag^+ ions to Ag^0 nanoparticles.

UV-Vis Spectroscopy: The optical properties of the synthesized AgNPs were characterized using a UV-Vis spectrophotometer (Shimadzu 1650 PC). The spectra were recorded between 300 nm and 700 nm, scanning at a resolution of 1 nm. The characteristic surface plasmon resonance (SPR) peak of AgNPs was observed at 430 nm.

Fourier Transform Infrared (FTIR) Spectroscopy: FTIR analysis was performed to identify functional groups involved in the reduction and stabilization of AgNPs. The dried sample was analyzed using an FTIR spectrometer (Shimadzu 8400s) over a wavelength range of 750 to 4000 cm^{-1} with a resolution of 1 cm^{-1}.

Transmission Electron Microscopy (TEM): The morphology and size of the synthesized AgNPs were analyzed using Transmission Electron Microscopy (TEM). Micrographs were captured to confirm the shape, size, and distribution of the nanoparticles. The synthesized AgNPs exhibited a sharp absorption peak at 430 nm, which is a characteristic feature of the surface plasmon resonance (SPR) of silver nanoparticles.

Bacterial cultures: The standard pathogenic bacterial cultures were procured from IMTECH, Chandigarh, India and used in the present study (Table 1). The bacterial cultures were rejuvenated in Mueller- Hinton broth (Hi-media laboratories, Mumbai, India) at 37^0C for 18h and then stocked at 4^0C in Mueller-Hinton Agar. The inoculum size of the bacterial culture was standardized according to the National committee for Clinical Laboratory Standards (NCCLS, 2002) guideline. The pathogenic bacterial culture was inoculated into sterile Nutrient broth and incubated at 37^0C for 3h until the culture attained a turbidity of 0.5 McFarland units. The final inoculum size was standardized to 10^5 CFU/mL with the help of SPC and Nephlo-turbidometer.

Antibacterial activity: Each bacterial strain was cultured in Mueller-Hinton Broth (MHB) at 37°C for 18-24 hours, and the turbidity was adjusted to 0.5 McFarland standard (approximately 1.5×1081.5 \times 10^81.5×108 CFU/mL). Mueller-Hinton Agar (MHA) plates were prepared, and each plate was uniformly swabbed with the respective bacterial inoculum. Wells of 6 mm diameter were punched into the agar, and 50 µL of the synthesized AgNP solution at different concentrations (e.g., 25 µg/mL, 50 µg/mL, 100 µg/mL) was added to each well. Sterile distilled water was used as a negative control, and standard antibiotics (e.g., ampicillin, ciprofloxacin) served as positive controls. The plates were incubated at 37°C for 24 hours, and the zones of inhibition around each well were measured using a caliper. The mean diameter of inhibition zones was calculated, and the results were compared with the controls to evaluate the antibacterial efficacy of the AgNPs. Each experiment was performed in triplicate to ensure accuracy and reproducibility. The extent of inhibition was categorized based on the diameter of the clear zones: strong inhibition (>15 mm), moderate inhibition (10-15 mm), and low or no inhibition (<10 mm).

Results and Discussion

The synthesis of silver nanoparticles (AgNPs) using *Lactobacillus plantarum* MTCC was confirmed through various characterization techniques. A visible color change in the cell filtrate from pale yellow to brown indicated the formation of AgNPs, which is a preliminary sign of nanoparticle synthesis due to surface plasmon resonance (SPR) of silver. UV-Vis spectroscopy further confirmed the synthesis, showing a characteristic absorption peak at 430 nm, corresponding to the SPR of AgNPs. The sharp peak suggests the formation of well-dispersed nanoparticles with uniform size distribution. Fourier Transform Infrared (FTIR) spectroscopy identified functional groups involved in the reduction and stabilization of AgNPs. The FTIR spectra displayed peaks corresponding to proteins and biomolecules, indicating their role as capping and stabilizing agents. Transmission Electron Microscopy (TEM) revealed the morphology of the synthesized AgNPs, showing spherical particles with an average size ranging from 10 to 30 nm. The uniformity in size and shape confirmed the efficiency of *Lactobacillus plantarum* in synthesizing stable AgNPs.

Characterization of silver nanoparticles: After UV – Visible spectrophotometer, further characterization was carried out by scanning electron microscopy, transmission electron microscopy and X – ray diffraction pattern generated by transmission electron microscope. On analysis, Spherical silver nanoparticles were observed and the size of silver nanoparticles synthesized by *Lactobacillus plantarum* 38–42 nm.

The silver nanoparticles were found to be capped by sodium citrate. On X – ray diffraction pattern generated by transmission electron microscope, it was confirmed that the silver nanoparticles are crystalline in nature.

Antibacterial Activity: The antibacterial activity was determined using the well diffusion method, measuring the zone of inhibition (ZOI) around the wells containing AgNPs. The results are summarized in the table below:

The antibacterial activity of the synthesized silver nanoparticles (AgNPs) was evaluated against both Gram-positive and Gram-negative bacteria. The results indicated that *Staphylococcus aureus* exhibited the highest susceptibility, with a zone of inhibition (ZOI) of 21 ± 1.8 mm, followed by *Staphylococcus epidermidis* with a ZOI of 18 ± 1.5 mm.

This higher susceptibility of Gram-positive bacteria is consistent with findings reported by Li *et al.,* (2019), who demonstrated that AgNPs are more effective against Gram-positive strains due to their thicker peptidoglycan layer that allows for easier penetration and disruption of the cell wall structure (Table 2).

Table.1 Bacterial cultures used in study (IMTECH, Chandigarh, India).

Bacterial Pathogens	MTCC Number
Proteus vulgaris	**426**
Staphylococcus epidermidis	**435**
Staphylococcus aureus	**96**
Escherichia coli	**739**
Pseudomonas aeruginosa	**424**
Klebsiella pneumoniae	**109**
Salmonella typhi	**733**
Enterobacter aerogenes	**111**
Salmonella typhimurium	**98**

Table.2 Antibacterial activity of synthesized nanoparticle against bacterial pathogens

Bacterial Strain	Zone of Inhibition (mm)
Proteus vulgaris	14 ± 1.2
Staphylococcus epidermidis	18 ± 1.5
Staphylococcus aureus	21 ± 1.8
Escherichia coli	16 ± 1.3
Pseudomonas aeruginosa	12 ± 1.0
Klebsiella pneumoniae	15 ± 1.2
Salmonella typhi	17 ± 1.4
Enterobacter aerogenes	13 ± 1.1
Salmonella typhimurium	16 ± 1.3

Figure.1 UV – Visible spectrophotometer graph of silver nanoparticles synthesized by *Lactobacillus plantarum* showed adsorption peak at 430 nm.

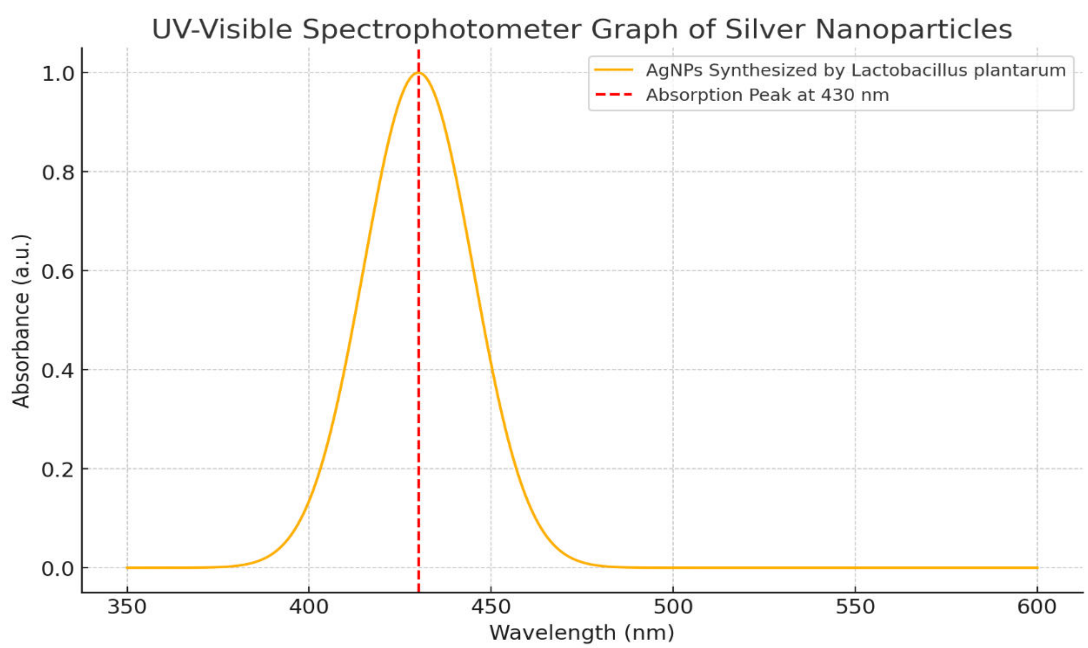
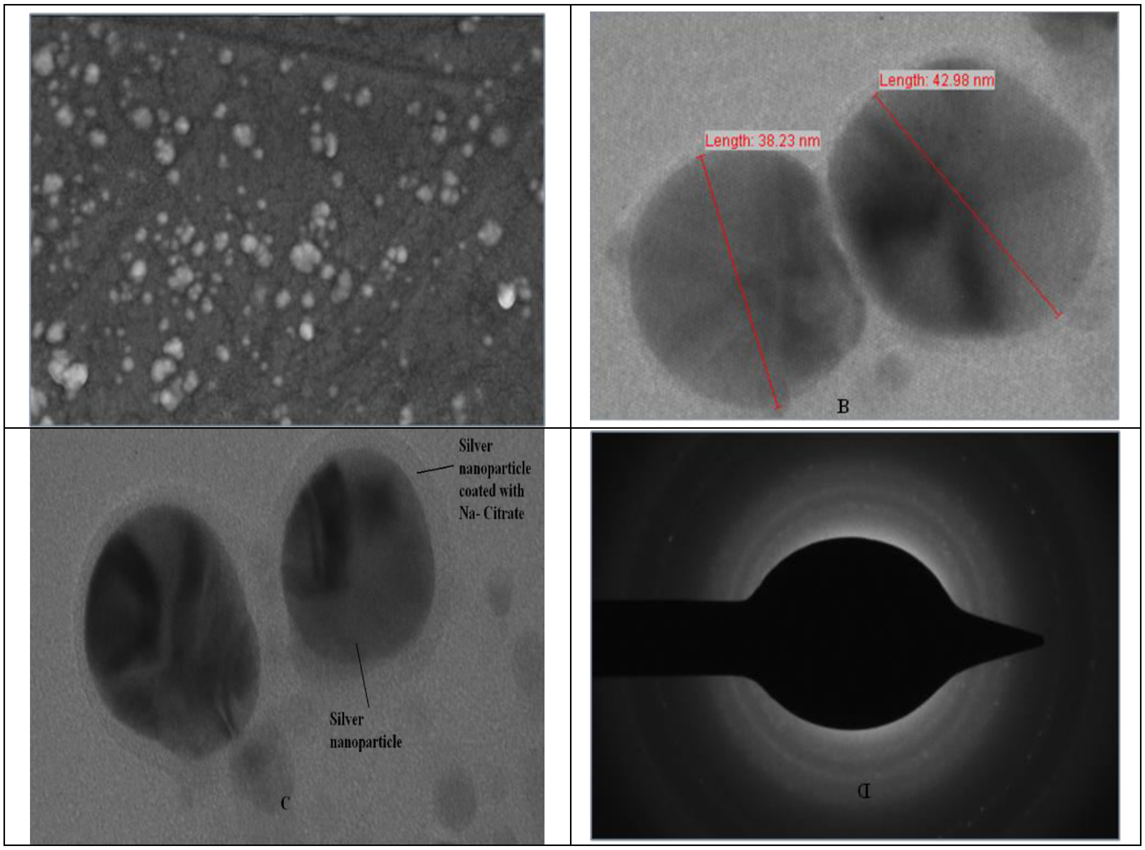


Figure.2 Showing SEM, TEM and XRD images of silver nanoparticles synthesized by *Lactobacillus plantarum* (*A*: SEM images, *B* and *C*: TEM images, *D*: XRD image).



In contrast, Gram-negative bacteria such as *Escherichia coli* and *Salmonella typhi* displayed moderate inhibition zones (16 ± 1.3 mm and 17 ± 1.4 mm, respectively). The outer membrane of Gram-negative bacteria acts as a protective barrier, making these strains more resistant to AgNPs. This observation aligns with the results reported by Morones *et al.,* (2005), who noted that the outer membrane of Gram-negative bacteria limits the permeability of nanoparticles, reducing their efficacy compared to Gram-positive strains.

Interestingly, *Pseudomonas aeruginosa* exhibited the lowest susceptibility (12 ± 1.0 mm), which may be attributed to its intrinsic resistance mechanisms, such as efflux pumps and biofilm formation. Similar findings were reported by Shrivastava *et al.,* (2007), who found that *Pseudomonas aeruginosa* shows higher resistance to nanoparticles due to its robust cell envelope and adaptability.

The moderate antibacterial activity against *Klebsiella pneumoniae* (15 ± 1.2 mm) and *Enterobacter aerogenes* (13 ± 1.1 mm) can also be explained by their ability to produce protective capsules, which hinder nanoparticle penetration (Guzman *et al.,* 2012). Moreover, the activity against *Proteus vulgaris* (14 ± 1.2 mm) and *Salmonella typhimurium* (16 ± 1.3 mm) demonstrates the broad-spectrum efficacy of AgNPs, corroborating findings by Lara *et al.,* (2011), who highlighted the potential of AgNPs as effective antimicrobial agents against both motile and non-motile bacterial strains.

The mechanisms underlying this antibacterial activity include AgNPs' ability to generate reactive oxygen species (ROS), disrupt cell membranes, and bind to cellular proteins and DNA, ultimately inhibiting bacterial growth and replication (Rai *et al.,* 2012).

The results of this study are in agreement with these proposed mechanisms and further validate the efficacy of AgNPs as potential antibacterial agents. Future research should focus on optimizing the size and concentration of AgNPs to enhance their efficacy and overcome resistance mechanisms in Gram-negative bacteria.

The study of silver nanoparticles (AgNPs) synthesized by *Lactobacillus plantarum* demonstrates their potent antibacterial activity against a range of Gram-positive and Gram-negative bacteria. Given their effective antimicrobial properties, AgNPs hold significant potential for various biomedical applications, such as wound dressings, antibacterial coatings, drug delivery systems, and environmental remediation. Future research should focus on optimizing the synthesis process, understanding resistance mechanisms, and evaluating the long-term safety of AgNP applications in clinical and industrial settings.

Author Contributions

Shinde Inamdar Sangita Abhaysinh: Investigation, formal analysis, writing—original draft. Samadhan R. Waghmode: Validation, methodology, writing—reviewing. Sheryas S. Shelar:—Formal analysis, writing—review and editing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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