



Biogenic synthesis of Silver nanoparticles using Secondary metabolites of *Lactiplantibacillus plantarum* and its potential applications

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Abstract



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This study investigated the eco-friendly synthesis of silver nanoparticles (AgNP's) using secondary metabolites derived from probiotic bacteria naturally existing in the human gut, such as *Lactiplantibacillus plantarum*. Probiotics are well-known for producing a wide range of bioactive compounds, including peptides, organic acids, bacteriocins, and exopolysaccharides, which can act as reducing and capping agents in bio-nanoparticle synthesis. These metabolites serve dual functions: facilitating nanoparticle formation and making their biological functionality. The synthesized AgNP's were characterized using advanced techniques to determine their size, morphology, and surface chemistry. The study emphasizes their potent antimicrobial, antifungal, and anticancer properties against MCF-7 breast cancer cell lines. Antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*. Antifungal efficacy against *Aspergillus niger* and *Aspergillus flavus*, demonstrating promising applications in pharmaceuticals and healthcare. Additionally, the nanoparticles were tested for their ability to enhance textile materials by testing their antibacterial activity and durability, thus supporting their role in functional fabrics used in medical clothing, sportswear, and hygienic textiles, which was compared to the chemically synthesized nanoparticles to achieve higher biocompatibility, stability, and sustainability. This work highlights the untapped potential of probiotic microorganisms as a novel and renewable source for nanomaterial production and paves the way for interdisciplinary applications spanning medicine, agriculture, environmental remediation, and smart textiles.

Keywords: *Lactiplantibacillus plantarum*, Silver Nanoparticles, Antibacterial, Antifungal, Anticancer activity

1. INTRODUCTION

Nanotechnology is a diverse and integrative field that focuses on creating, developing, and utilizing materials at the atomic and molecular scale. At this scale, the physicochemical properties of nanomaterials differ significantly from their bulk counterparts due to their high surface area-to-volume ratio, leading to enhanced reactivity, improved mechanical strength, and unique optical behaviours. Among various nanomaterials, metal nanoparticles, particularly silver nanoparticles (AgNP's), have drawn considerable interest for their biomedical and industrial applications. Nanoparticles are ultra-small materials with dimensions in the nanometres range, making them highly versatile for various scientific and industrial applications. Their composition can vary, including metallic, polymeric, ceramic, and carbon-based nanoparticles. Among them, metal nanoparticles, especially AgNP's, have gained prominence due to their exceptional antimicrobial, anticancer, and drug delivery capabilities, attributed to their unique physical and chemical properties¹. Silver

nanoparticles are widely studied due to their potent antibacterial, antiviral, and antifungal properties. Their antimicrobial function primarily arises from the release of silver ions (Ag⁺), which exhibit toxicity towards various pathogens, disrupting cellular structures and leading to microbial death². Owing to their broad-spectrum antimicrobial efficacy, AgNP's have been utilized in wound dressings, topical creams, food packaging materials, and various consumer products³.

This bactericidal activity has positioned AgNP's as a viable alternative to conventional antibiotics, potentially mitigating the rising concern of antibiotic resistance⁴. However, chemical and physical synthesis methods, though effective in controlling particle characteristics, are often costly and environmentally harmful, producing undesirable by-products. In contrast, biological synthesis using microorganisms has emerged as a sustainable and eco-friendly alternative for nanoparticle production⁵.

Certain microorganisms, particularly bacteria, possess the ability to biosorb and bioaccumulate metal

ions, enabling their enzymatic reduction into nanoparticles⁶. Research indicates that bacteria from metal-rich environments demonstrate increased tolerance to metal ions, enhancing their efficiency in nanoparticle biosynthesis⁷. Among bacterial candidates, lactic acid bacteria (LAB) have proven effective in AgNP biosynthesis due to their non-pathogenic nature and common presence in fermented dairy and food products⁸. While studies utilizing LAB supernatants for AgNP biosynthesis have demonstrated the successful production of spherical nanoparticles, thereby simplifying downstream processing research employing LAB cell biomass for nanoparticle synthesis remains limited. *Lactobacillus plantarum* TA4, a probiotic strain isolated from Tapi pulut, which was evaluated for its ability to synthesize AgNP's using its cell biomass. This strain had previously exhibited zinc tolerance and was capable of ZnO nanoparticle synthesis. The research assessed the strain's tolerance to varying Ag⁺ concentrations and confirmed AgNP formation using UV-Vis spectroscopy, followed by characterization via dynamic light scattering (DLS), Fourier-transform infrared spectroscopy (FTIR), and high-resolution transmission electron microscopy (HR-TEM). The antimicrobial and antioxidant activities of the synthesized AgNP's were also examined¹⁰.

Silver nanoparticles have found extensive applications across multiple domains due to their versatile properties. In biomedical applications, they are used in wound dressings, drug delivery systems, topical creams, and antibacterial coatings to prevent infections⁹. In the food industry and packaging, they are integrated into food storage materials to inhibit microbial growth and enhance shelf life¹¹. In consumer goods, they are found in textiles, cosmetics, and personal care products for antimicrobial protection. In environmental protection, they are applied in water filtration systems and pollution control mechanisms due to their antimicrobial potency¹².

As scientific advancements continue, integrating probiotic microorganisms into AgNP biosynthesis holds the potential to enhance sustainable nanotechnology and broaden the biomedical and industrial applications of AgNP's. Furthermore, developing cost-effective and environmentally friendly synthesis methods will help in optimizing AgNP's production for widespread industrial adoption¹³.

2. MATERIALS AND METHODS

Collection of bacteria and subculturing:

The probiotic bacterial strain of *Lactobacillus plantarum* was procured from the Centre for Bioscience and Nanoscience Research, Coimbatore, Tamil Nadu, India, and subcultured in de Man, Rogosa and Sharpe (MRS) broth (Himedia, Mumbai), prepared by dissolving 55.15g of medium in 1000mL of distilled water and sterilizing at 121°C for 15 minutes. After cooling under aseptic conditions, the culture was inoculated into the medium and incubated at 37°C for 24 hours. Bacterial growth was confirmed by turbidity and the culture was used for further experiments.

Biomass estimation:

Biomass was estimated to identify the optimum production of metabolites by the bacteria. 1ml of the culture was centrifuged at 5000 rpm for 5 minutes. After centrifugation supernatant was removed and pellet was weighed and calculated the biomass using the following formula;

$$\text{Biomass} = \text{pellet with tube} - \text{empty tube}$$

Synthesizing of silver nanoparticle

The cell-free extract obtained after centrifugation was mixed with an equal volume of 1 mM silver nitrate solution (v/v) and incubated in flasks at 30 °C on a shaker at 70 rpm for 24 h. A visible colour change indicated the formation of silver nanoparticles (AgNP's). The reaction mixture was allowed to centrifuge at 10,000 rpm for 20 mins and the pellet of the nanoparticle sample was collected, which were washed thrice with distilled water to remove residual media components. The purified AgNP's were dried at 70 °C for 15 h and ground into fine powder for further characterization¹⁵.

Characterization study of the silver nanoparticle

UV-Vis spectroscopy

UV-vis spectrophotometer from 300 to 600 nm using UV-Visible spectrophotometer-Labtronics LT291, operated at a resolution of 1 nm was used as a function of wavelength for spectral analysis of silver nanoparticles¹⁶.

FT-IR spectroscopy

Fourier Transform Infrared (FTIR) Spectroscopy was employed to identify the functional groups responsible for the reduction of silver ions (Ag⁺) and the stabilization (capping) of biosynthesized silver nanoparticles by microbial extract¹⁵. The analysis was conducted using an FTIR spectrometer (Shimadzu) to detect biomolecular interactions indicative of nanoparticle formation and capping efficiency.

Precipitation and purification of compounds

The media was centrifuged at 5000rpm for 5 minutes and the cell free extract was collected. The cell free extract was precipitated with 60% of ammonium sulphate solution. For this precipitation 20 ml of the sample was mixed with 12gm of the ammonium sulphate and mixed well, this was incubated at -40C for overnight. The incubated sample was centrifuged at 10000 rpm for 7minutes and the pellet was used for further study¹⁷.

Thin Layer Chromatography

TLC method was used for the identification of the secondary metabolites. The chromatographic sheet was set with 5cm width and 8cm length and spotted the sample 1cm above from the bottom using capillary tubes (about 50 µl). Sample placed and this was run in solvent system of N-Butanol: methanol, water, acetic acid (5:2.5:2:1). After 1cm from the top of the plate this was taken out from the solvent and dried to visualise the compound, the same method was used for the TLC

study¹⁴. R_f value was calculated after visualisation of the compound as spot in plate. R_f value calculation formula is;

$$R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Anti textile activity

The antibacterial efficacy of the sample was assessed through textile-based diffusion. A sterile gauze cloth (0.5×0.5mm) was coated with 100µL of the test sample and placed on Mueller Hinton agar (39g/L), which had been sterilized at 121°C for 15minutes and poured into Petri plates for solidification. Following solidification, 70µL of bacterial cultures - *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* - were swabbed onto the agar surface using sterile cotton swabs. The sample-coated cloth was carefully positioned, and the plates were incubated at 37°C for 24hours. Antibacterial activity was evaluated by measuring the diameter of the zone of inhibition (in mm) around each cloth¹⁸.

Antifungal activity

The antifungal activity of the extracts was assessed using the agar well diffusion method. Fungal cultures of *Aspergillus niger* and *Aspergillus flavus* were inoculated at the sterile Petri dishes containing malt agar (45g/L), which had been sterilized at 121°C for 15 minutes and allowed to solidify. The fungal cultures were uniformly spread using sterile cotton swabs, and 6mm wells were created using a sterile cork borer. Each well received 50µL of the extract, and the plates were incubated at 30°C for 2-5 days. Antifungal efficacy was determined by measuring the diameter of the inhibition zone. Fluconazole disc acted as the positive control, while the crude sample served as the negative control¹⁹.

Anti - Cancer Activity:

MCF-7 breast cancer cell lines, obtained from the National Centre for Cell Sciences (Pune, India), were maintained at the Centre for Bioscience and Nanoscience Research, Eachanari, Coimbatore. The cells were cultured in DMEM medium supplemented with sodium carbonate, glucose, and 10% BSA. After seeding in T-flasks, they were incubated in a CO₂ incubator at 37°C, pH 7–7.5, and 70–80% humidity for 24–72 hours. Cell growth was verified under an inverted microscope and used for further assays.

For the MTT assay, cells were seeded in 96-well plates and allowed to adhere for 24 hours under similar incubation conditions. Various concentrations of silver nanoparticles were introduced, along with DMSO as the blank and untreated cells as the control. After 24 hours of treatment, cells were rinsed with DMSO and trypsin. Then, 20µL of MTT dye was added to each well, gently mixed, and incubated again at 37°C for 24 hours. The resulting purple formazan product was measured at 570nm using a 96-well ELISA reader (Robonik, India), and cell viability (%) was calculated accordingly²⁰.

$$\% \text{ of cell viability} = \left[\frac{\text{Mean abs of treated cells}}{\text{Mean abs of Untreated cells}} \right] \times 100$$

3. RESULTS AND DISCUSSION

Collection, subculturing and Biomass estimation:

This research presents an innovative and valuable advancement in the field of nanotechnology through the biogenic synthesis of silver nanoparticles (AgNP's) using secondary metabolites from *Lactiplantibacillus plantarum*. The *Lactobacillus plantarum* culture was initially subcultured in MRS broth and incubated at 37°C for 24 hours to promote growth. The growth characteristics were observed and documented in Figure 1. Biomass estimation was carried out to assess the culture's ability to synthesize and produce metabolites effectively. This step is crucial for evaluating the potential of *Lactobacillus plantarum* as a viable product to synthesis nanoparticle for industrial and biotechnological applications²¹.

Thin Layer Chromatography:

A key distinguishing feature is the application of thin layer chromatography (TLC) to isolate and confirm the metabolites responsible for nanoparticle synthesis, offering a level of the biochemical insight that is often used in comparable studies. Upon extracting their components, the biomass is subjected to thin layer chromatography. The biomass is separated, identified and purified using the TLC as shown in figure 2 and the respective R_f values are calculated accordingly¹⁴.

Precipitation and purification of compounds:

The media was centrifuged at 5000rpm for 5minutes and the cell free extract was collected. The cell free extract was precipitated with 60% of ammonium sulphate solution. For this precipitation 20 ml of the sample was mixed with 12gm of the ammonium sulphate and mixed well, this was incubated at -4°C for overnight. The incubated sample was centrifuged at 10000 rpm for 7minutes and the pellet was used for further study.

Synthesizing of silver nanoparticles:

In line with the current trends that emphasize eco- friendly synthesis methods - similar to the approaches done by Khan *et al.*¹ and Karunakaran *et al.*⁵ - this study highlights a sustainable technique for nanoparticle production. The silver nanoparticles were produced using the silver nitrate and the visible colour change from yellow to brown takes place in the sample and is shown in the Figure 3.

Characterization study of the silver nanoparticle:

The silver Nanoparticle sample was characterized using the UV-Vis spectroscopy and the UV-visible absorption spectra of the silver nanoparticles exhibited prominent peaks between 420 and 450 nm, which is attributed to surface plasmon resonance²², the similar observation and results obtained here also the peak was at 430nm. Figure 3 showing the current existed results.

Figure 1(Left) - Subculturing of *Lactobacillus* bacteria in MRS brothFigure 2 (Middle) - TLC of the Biomass collected from *Lactobacillus* bacteria.

Figure 3 (Right) - Silver nanoparticles produced compared with the control sample

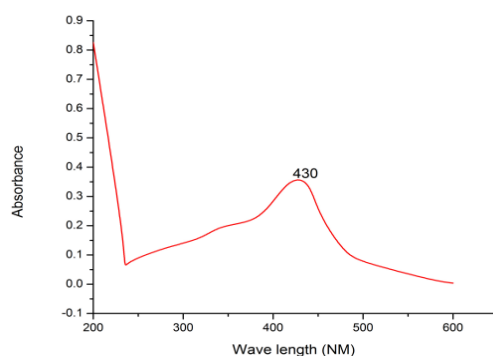


Figure 4: UV Vis - Peak at 430nm confirms the presence of silver nanoparticle

FTIR analysis confirmed key functional groups and bonding interactions involved in the biosynthesis and stabilization of silver nanoparticles using *Lactobacillus plantarum*. Probiotic-derived capping agents and surface proteins supported eco-friendly nanoparticle formation. These results validate synthesis success and expand potential applications across diverse scientific fields.^{15,16}

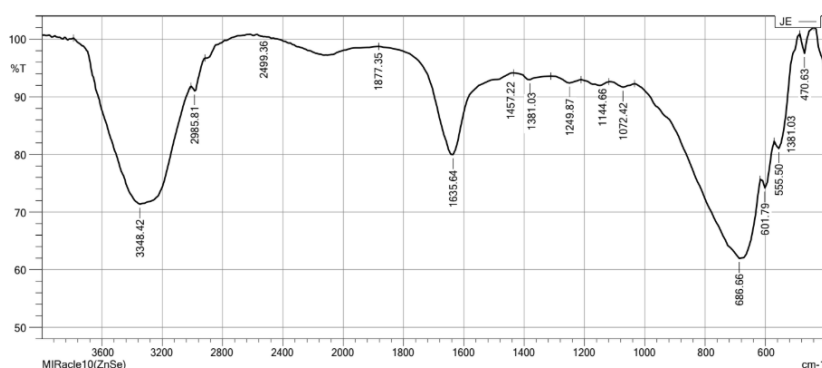


Figure 5: FTIR Results - Effective functional groups of Silver Nanoparticles

S.No	Frequency	Functional group
1	3348.42	Alcohol-OH stretch
2	2985.81	Carboxylic acid OH stretch
3	2499.36	C≡N stretch
4	1877.35	C=O anhydride
5	1635.64	C=C alkene
6	1457.22	CH ₂ Bend
7	1381.03	C-F strong bond
8	1249.87, 1144.66	C-O-C stretch
9	1072.42	C-OH stretch
10	686.66	C-Cl
11	601.79	C-Cl strong bond
12	555.50, 470.63	C-Br strong bond

Anti-textile and Antifungal activity

The anti-textile activity of the silver nanoparticle is done using the Gauss cloth against the various pathogenic bacteria species including *E.coli*, *S.aureus*, *K.pneumoniae* where the crude acts as the negative control and Antibiotic disc of Ceftazidime acts as the positive control. This activity serves as one of the applications of silver nanoparticles in medical field²³. The result of the anti-textile activity of silver nanoparticle is tabulated in the Table 1.

Table 1: Inhibition of silver nanoparticles coated on textile against pathogenic bacteria

Microorganism used	Zone of inhibition (mm)			
	Crude	NP ₅₀	NP ₁₀₀	Disc Caz)
<i>E. coli</i>	-	24	19	28
<i>S.aureus</i>	-	19	23	30
<i>K.pneumoniae</i>	-	22	34	29

Antifungal activity was investigated against *Aspergillus niger* and *Aspergillus flavus*. The presence of inhibition zones around the treatment site signified antifungal potency. Fluconazole antibiotic served as the positive control, while the crude sample functioned as the negative control was tabulated in Table 2.

Table 2- Inhibition by silver nanoparticles against spore-forming fungi

Microorganism used	Zone of inhibition (mm)		
	Crude	NP	Antibiotic
<i>Aspergillus niger</i>	12	15	30
<i>Aspergillus flavus</i>	11	19	22

The antimicrobial results are particularly essential, with a substantial 34 mm inhibition zone against *Klebsiella pneumoniae*, surpassing findings from previous works like those of Yusof *et al.*¹⁰ A unique element of this research is the integration of AgNP's into textile materials to enhance their anti-bacterial properties - an application not commonly explored in earlier publications. Additionally, the study assesses the antifungal capabilities of the nanoparticles

Anti-cancer activity:

To evaluate the anticancer efficacy of the synthesized silver nanoparticles, the MCF-7 human breast cancer cell line was employed as the in vitro model system. Upon achieving approximately 80–90% confluence, the cells were exposed to various concentrations of silver nanoparticles, prepared in serial dilutions to observe dose-dependent effects. Following a 24-hour incubation period, cellular metabolic activity was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. MTT dye was added to each well and incubated, allowing viable cells to reduce the yellow tetrazolium salt to purple formazan crystals, which were subsequently solubilized using

DMSO. The intensity of the developed colour, directly proportional to the number of viable cells, was measured spectrophotometrically at 570 nm. The percentage of cell viability was calculated relative to untreated control cells, and the data were compiled and represented in Table 3 and figure 6 to elucidate the cytotoxic profile of the silver nanoparticles.

Table 3: Cell viability results of the synthesised nanoparticle against MCF 7 Cell Line

Concentration of sample used (µl)	% of cell viability
2	83.45
4	79.36
6	77.08
8	72.38
10	66.16

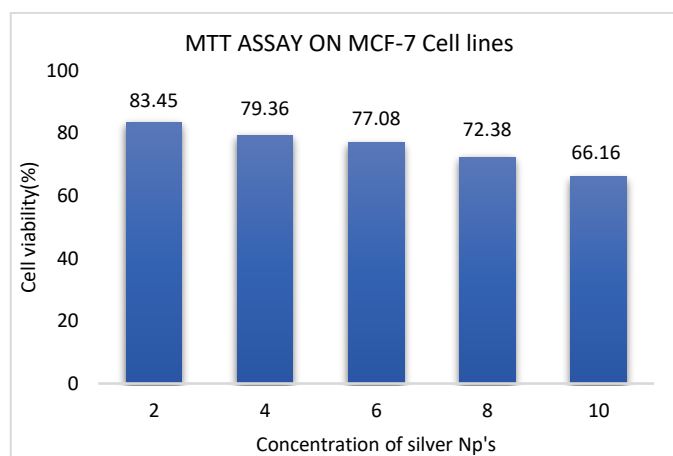


Figure 6: MTT Assay on MCF-7 Breast cancer cell lines

Cytotoxic potential against MCF-7 breast cancer cells using the MTT assay, showing promising results. These multifaceted evaluations contribute to the study's originality. Altogether, this work not only reinforce the significances of biologically synthesized AgNP's but also broadens their application potential in areas such as healthcare, textiles, and antimicrobial product development^{24,25}.

5. CONCLUSION

Synthesised nanoparticles (AgNPs) were characterised using advanced analytical techniques such as UV-Visible and FTIR to assess their conversion into nanoparticles and functional groups for the primary confirmation of nanoparticles. This study highlights their significant antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*, and antifungal activity against *Aspergillus niger* and *Aspergillus flavus*. The anticancer activity against MCF-7 cell lines shows promising results and which demonstrating the importance and applications of nanoparticle with bacterial extract for pharmaceuticals and healthcare.

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