International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 19s, 2025
https://theaspd.com/index.php

"Biosynthesis Of Silver Nanoparticles Using Isolated Lactobacillus Delbrueckii And The Evaluation Of Its Antioxidant, Antibacterial And Anticancer Property"

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Abstract
This study focuses on the green synthesis of silver nanoparticles (AgNPs) using Lactobacillus delbrueckii isolated from yogurt, and the evaluation of their antioxidant, antibacterial, and anticancer properties. The bacterial strain was identified through Gram staining, biochemical tests, and confirmed by 16S rRNA sequencing. The cell-free supernatant of L. delbrueckii was mixed with silver nitrate, leading to the formation of AgNPs, as indicated by a visible colour change from pale yellow to dark brown. The synthesized nanoparticles were characterized using UV-Vis spectroscopy, which showed a peak at 415 nm, FTIR analysis revealed functional groups involved in nanoparticle stabilization, and FE-SEM confirmed that the particles were spherical with an average size of 21.47 nm. The AgNPs exhibited notable antioxidant activity in the DPPH assay (IC_{50} ≈ 100.42 μg/ml) and showed antibacterial effects against common pathogens such as Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Pseudomonas aeruginosa. In addition, the AgNPs demonstrated significant anticancer activity against HCT-116 human colorectal carcinoma cells, with an IC_{50} of 85.44 μg/ml. These results suggest that L. delbrueckii-mediated silver nanoparticles have promising potential for biomedical applications. However, further studies are needed to optimize the synthesis process and assess their long-term safety and therapeutic efficacy.

INTRODUCTION

Lactic acid bacteria (LAB) have been traditionally consumed through fermented foods like dairy products. They are currently the focus of extensive global research due to their essential role in producing antimicrobial substances and include potential antitumor properties. Nanoparticles are being utilized for a variety of applications, ranging from medical treatments to industrial uses. The biosynthesis of nanoparticles by microorganisms is an environmentally friendly and sustainable technology.

Silver nanoparticles (AgNPs) are highly promising in nanomedicine due to their inhibitory and bactericidal properties. Silver nanoparticles have also been applied to different kinds of cancer lines, and it has been found that they suppress the sickness and tumor growth without harming healthy cells. The silver nanoparticles synthesized from the Lactobacillus sp isolated from the yoghurt sample is effective against the antioxidant, antibacterial and anticancer activity.

Isolation and identification of the Lactobacillus sp

The bacterial strain was isolated using spread plate and streak plate method. The isolated bacterial strain was identified by using Gram staining and biochemical tests like IMViC, urease tests. Then the results were obtained and the species identification of the genus Lactobacillus was identified by using the molecular technique 16S rRNA sequencing.

Preparation of cell-free supernatant of isolated Lactobacillus strains

The MRS broth was prepared for mass culture and the Lactobacillus strains were inoculated into the broth. The bacterial culture was centrifuged at 8000 rpm for 10 minutes at the temperature range of 25°C. The supernatant was transferred into the sterile capped test tubes and used for the synthesis of silver nanoparticles.

Synthesis and characterization of silver nanoparticles

The prepared silver nitrate solution ($AgNO_3$) was added to the Lactobacillus sp cell-free supernatant sample and mixed well. The results were observed. The characterization and identification of the synthesized silver nanoparticles were determined by the following methods like UV-vis spectrophotometer, FTIR and FE-SEM analysis.

DPPH free radical scavenging activity

A 0.4 mm solution of DPPH in methanol was prepared and 2 ml of this solution was added to different concentrations of the biosynthesized silver nanoparticles sample ranging from 100, 150, 200, 250 and 300 µl and incubated at dark room temperature for 15 minutes. The quercetin was added as the standard. The results were recorded and the colour change was observed.

Antibacterial activity using agar well diffusion method

The bacterial cultures S. aureus, B. subtilis, E. coli and P. aeruginosa were swabbed evenly over the agar surface of the prepared MHA plates. The wells were punched in the solidified agar and the sample was loaded in different concentrations as followed: 25, 50, 75 and 100 µl into each well. The plates were incubated for 12-14 hours. After incubation, the zone of inhibition was observed .

Anticancer activity

The anticancer activity of the biosynthesized silver nanoparticles for sample was tested against HCT 116(Human colorectal carcinoma cell line) cell line by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. The Percentage of cell viability was calculated using the formula,

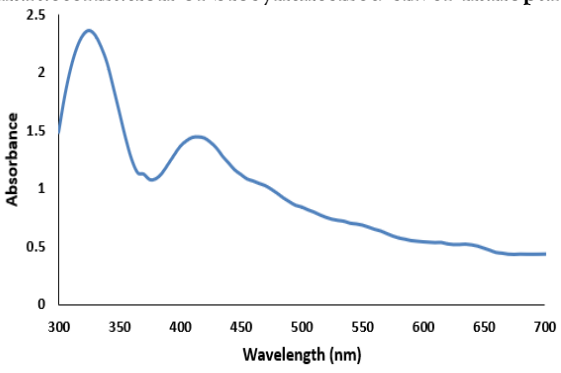
Cell viability (%) = (Absorbance of sample/Absorbance of control) × 100.

RESULTS

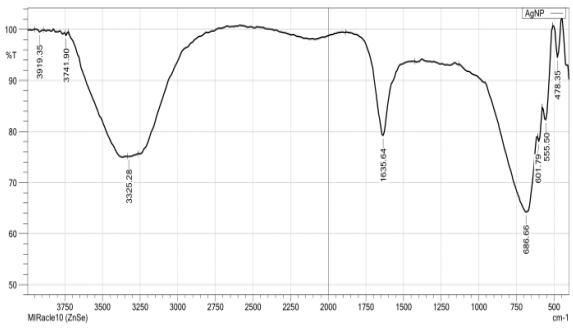
Identification of the Lactobacillus sp

Small, creamy to white, round and mucoid colonies were observed in the streak plate method. The Gram staining result indicated that the bacterial culture showed purple colour, rod-shaped, gram-positive, non-spore forming bacteria. The biochemical tests involved IMViC and urease tests showed negative result indicated no colour change. The organism was identified as Lactobacillus sp. The species identification was identified by the molecular technique called 16s rRNA sequence. The sequencing data were analyzed and compared with reference sequences from the GenBank database. This confirmed the presence of Lactobacillus delbrueckii in the isolated colony of the yoghurt sample.

Characterization of biosynthesized silver nanoparticles:



UV-vis absorption spectra



FTIR analysis

The Lactobacillus delbrueckii treated with $AgNO_3$ solution was observed for colour changes from pale yellow to dark brown colour after incubation. This colour change indicated the reduction of silver ions (Ag^+) to silver nanoparticles (AgNPs). UV-Vis spectroscopy analysis showed a distinct absorption peak at 415nm, confirmed the successful synthesis of silver nanoparticles through the reduction of silver ions. In FTIR analysis, the absorption peaks were observed that ranged from 3919.35 cm-1 to 478.35 cm-1. The functional groups identified in the spectrum suggested that obtained alkyne, amide and alcohol from the Lactobacillus delbrueckii contributed to the successful formation and stabilization of the silver nanoparticles.

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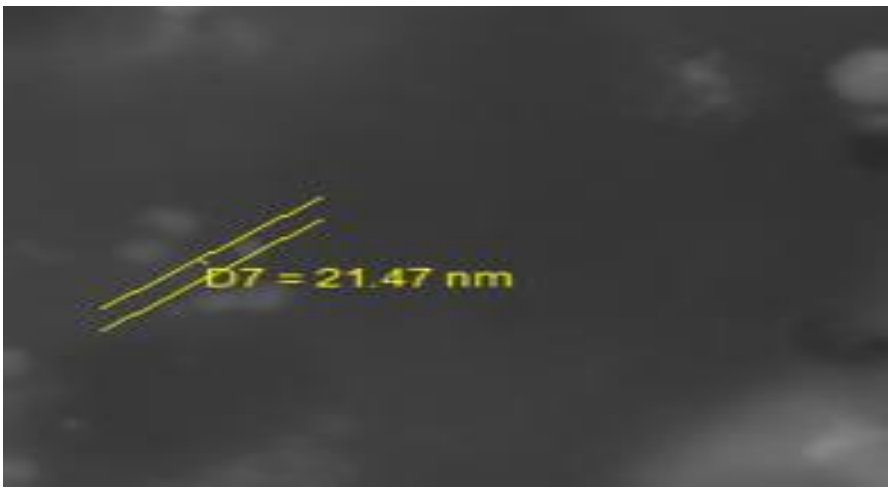
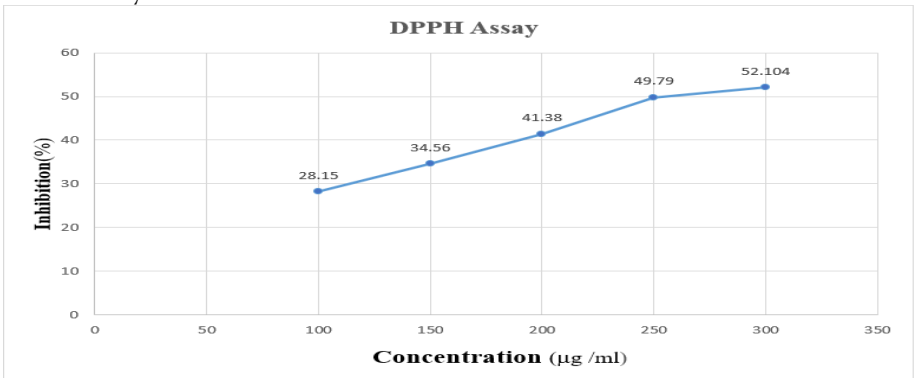


FE- SEM image

The SEM analysis confirmed that the silver nanoparticles synthesized by the Lactobacillus delbrueckii were spherical, with size ranged 21.47 nm

. Antioxidant activity

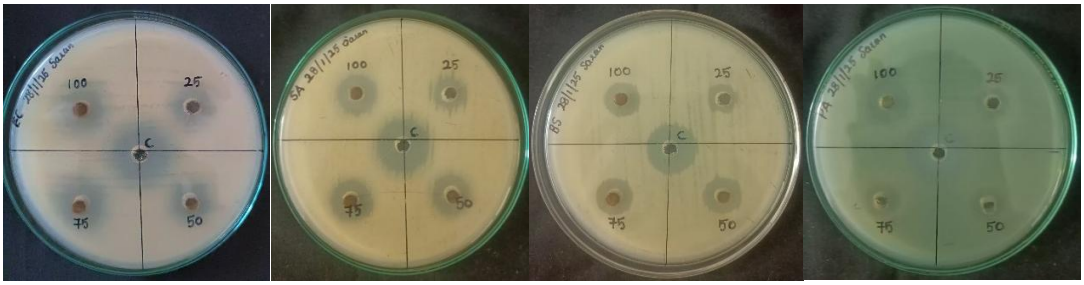
DPPH assay



Antioxidant assay using DPPH method

- The colour change was observed after incubation. The percentage of inhibition exhibited by biosynthesized silver nanoparticle was 49.75% at 250µl. This showed the presence of antioxidant activity. However, the inhibition percentage of the standard quercetin was found to be greater than the silver nanoparticle suspension at 50µg/ml (48.45%). The IC50 value of the sample was calculated by using the formula,
- IC50 = (100×50) ÷ 49.75 (nearest value of 50% inhibition)
- IC50 = 100.421 µg/ml

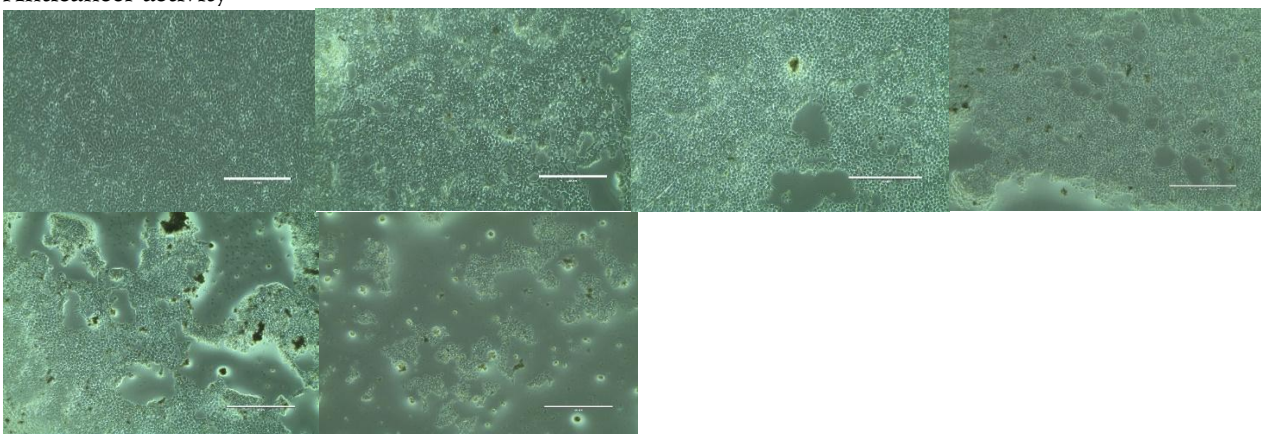
Antibacterial activity (agar well diffusion method)

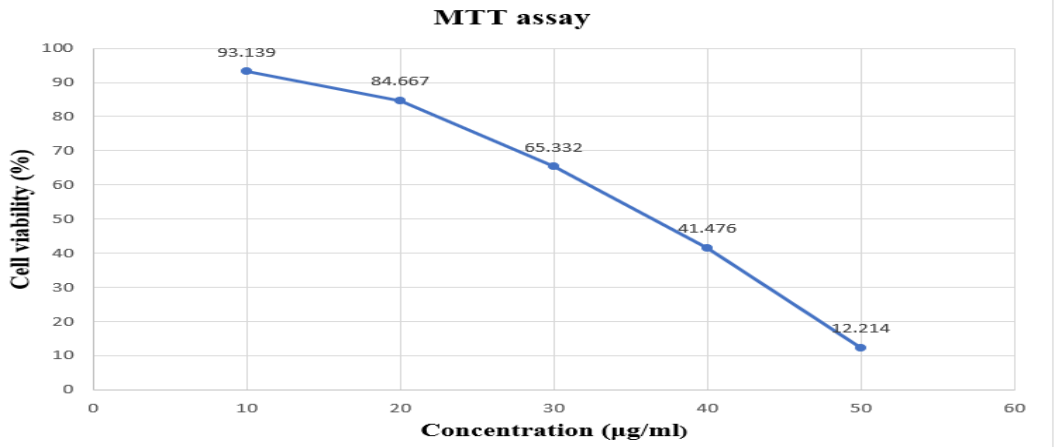


S. No	Name of the microorganism	Zone of inhibition in diameter (mm)				
		Positive control	25 (µg/ml)	50 (µg/ml)	75 (µg/ml)	100 (µg/ml)
1.	*Escherichia coli*	28	15	17	20	24
2.	*Staphylococcus aureus*	22	13	15	15	16
3.	*Bacillus subtilis*	19	10	14	14	15
4.	*Pseudomonas aeruginosa*	22	12	13	14	15

The biosynthesized silver nanoparticles showed the significant antibacterial activity against the pathogenic microorganisms. The zone of inhibition of each concentration of the sample was measured and tabulated. However, the positive control ampicillin, exhibited the higher zone of inhibition compared to the sample.

Anticancer activity





The treatment of HCT 116 cells with the silver nanoparticle suspension resulted in significant cytotoxic effects on cancer cell viability, as a huge majority of the cells were found to be destroyed after treatment. After calculation, the sample concentration needed to produce a 50% reduction (IC50) was determined to be 85.44 µg/ml. The cell viability was calculated using the values of average and control as follows:
Cell viability % = (Absorbance of sample / Absorbance of Control) × 100

CONCLUSION

In this present study, the yoghurt sample was used for the isolation of Lactobacillus delbrueckii which was used for the biosynthesis of silver nanoparticles. The biosynthesized silver nanoparticles showed the presence of antioxidant activity. The biosynthesized nanoparticles exhibited the presence of antibacterial activity against a range of test pathogens. The silver nanoparticles were effective in reducing the viability of cancer cells against human colorectal carcinoma cell line (HCT 116) and this exhibited the presence of anticancer activity. However, further studies are needed to optimize the synthesis process, characterize the nanoparticles in detail, and evaluate their long-term stability and safety for various applications.