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Sustainable biosynthesis of silver nanoparticles using *Streptomyces diastaticus* cultivated in apple pomace and evaluation of their antibacterial activity

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Abstract

The increasing demand for environmentally sustainable nanomaterials has led to the development of green synthesis approaches using biological sources and agro-industrial waste. In the present study, silver nanoparticles (AgNPs) were biosynthesized using extracellular metabolites produced by *Streptomyces diastaticus* cultivated in apple pomace. Apple pomace served as a low-cost fermentation substrate for metabolite production, thereby integrating waste valorization with green nanotechnology. The synthesized AgNPs were confirmed by a visible colour change from reddish to dark olive-brown following incubation with silver nitrate solution. UV-Visible spectroscopy revealed a characteristic surface plasmon resonance (SPR) peak at 410 nm, confirming AgNP formation. Fourier transform infrared (FTIR) spectroscopy demonstrated the presence of functional groups associated with extracellular biomolecules involved in the reduction, capping, and stabilization of the AgNPs. X-ray diffraction (XRD) analysis confirmed the crystalline nature of the synthesized nanoparticles, while transmission electron microscopy (TEM) revealed predominantly quasi-spherical nanoparticles with an average particle size of 16.6 ± 4.49 nm. The antibacterial activity of the synthesized AgNPs was evaluated against *Escherichia coli*, *Klebsiella pneumoniae*, and *Shigella flexneri* using the agar well diffusion method. The nanoparticles exhibited antibacterial activity, with the highest inhibition zone (16 mm) being observed against *K. pneumoniae* at a concentration of 100 μ L. These findings demonstrate that apple pomace is an effective and sustainable substrate for the growth of *S. diastaticus*, metabolite production, and AgNP synthesis. This study highlights the potential of integrating agro-waste valorization with microbial nanotechnology for the sustainable production of biologically synthesized AgNPs with promising antibacterial applications.

Keywords: Silver nanoparticles, *Streptomyces diastaticus*, apple pomace, green synthesis, agro-industrial waste, antibacterial activity

1. Introduction

Green nanotechnology has emerged as an environmentally sustainable approach for the synthesis of silver nanoparticles (AgNPs) using biological sources such as plants, algae, fungi and bacteria [1]. Compared to other AgNPs synthesis methods such as physical and chemical methods, biological method is cost-effective, environmentally friendly, and do not leave harmful by-products in the environment. Hence, green synthesis of AgNPs has attracted considerable interest as the process aligns with sustainable development principles and environmental protection.

Various biological agents have been used for AgNPs synthesis; *Streptomyces* species are of particular interest due to their ability to synthesize various secondary metabolites, enzymes, proteins, and other bioactive compounds which can facilitate the reduction of silver ions to form AgNPs while also playing a part in the stabilization and capping of the nanoparticles [2]. The biomolecules influence the stability, size, and morphology and therefore do not necessitate the need for hazardous chemicals for the reduction process. Since *Streptomyces* species have the ability to synthesize a wide range of bioactive compounds, they remain useful for the green synthesis of the nanoparticles [3].

Silver nanoparticles (AgNPs) possess unique physicochemical properties, including a high surface-area-to-volume ratio, excellent electrical conductivity, and broad-spectrum antimicrobial activity [4].

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These properties have led to their widespread applications in medicine, environmental remediation, food preservation, agriculture, and biomedical engineering. Among these applications, their antimicrobial potential has attracted considerable attention because of the increasing global burden of antimicrobial resistance (AMR). The rapid emergence and spread of AMR have reduced the effectiveness of conventional antibiotics, creating an urgent need for alternative antimicrobial agents [5]. Unlike conventional antibiotics that target a single cellular process, AgNPs exert antimicrobial effects through multiple mechanisms, including disruption of the bacterial cell membrane, generation of reactive oxygen species, interaction with proteins and DNA, and inhibition of metabolic pathways, thereby reducing the likelihood of resistance development [4].

The efficiency and biological activity of AgNPs using secondary metabolites of *Streptomyces* species is influenced by the composition of the fermentation media used for the growth of the specific *Streptomyces* species. It also determines the quantity and diversity of the metabolites that are involved in AgNPs synthesis. Due to the increasing environmental pollution concerns of agro-wastes and the need to find low-cost fermentation media, current research has reported the use of agro-industrial wastes as substrates for microbial cultivation and metabolite production. These residues serve as low-cost nutrient sources while contributing to the management of agricultural waste and reducing environmental pollution [6]. Several studies have demonstrated the successful use of agro-waste materials, including sugarcane bagasse, coconut oil cake, and groundnut shells, for microbial fermentation and the production of valuable metabolites [7].

Apple pomace, the major by-product of the fruit juice processing industry, is generated in large quantities worldwide and is often discarded as waste, contributing to environmental pollution. However, it contains carbohydrates, dietary fibre, proteins, vitamins, minerals, and phenolic compounds that make it a suitable substrate for microbial growth and metabolite production [8]. The utilization of apple pomace as a fermentation substrate therefore provides an environmentally sustainable strategy for waste valorization while reducing the cost of microbial bioprocesses.

Although several studies have reported the biosynthesis of silver nanoparticles using plant extracts and microbial cultures, only limited studies have investigated the use of agro-industrial waste-based fermentation media for the cultivation of *Streptomyces* species for nanoparticle synthesis. Furthermore, the use of apple pomace as a sustainable substrate for the cultivation of *Streptomyces diastaticus* and the subsequent biosynthesis of AgNPs has not been adequately explored. Therefore, the present study addresses this research gap by utilizing apple pomace as a low-cost and environmentally sustainable substrate for the production of extracellular metabolites involved in AgNP synthesis.

The novelty of the present work lies in the integration of agro-waste valorization with green nanotechnology through the cultivation of *Streptomyces diastaticus* in apple pomace medium for the biosynthesis of silver nanoparticles. In addition, the antibacterial activity of the synthesized nanoparticles was evaluated against selected human

pathogens to determine their potential biomedical applications.

2. Materials and Methods

2.1 Selection of bacterial strain

A previously isolated strain of *Streptomyces diastaticus* (Genbank accession number: PQ469050.1) was selected for the biosynthesis of AgNPs. The isolation, identification and optimization of its growth conditions have been described in our previous study.

2.2 Use of Apple pomace for production of secondary metabolites

Apple pomace was collected from local fruit juice vendors in Vadavalli area, Coimbatore Tamil Nadu. The procedures for processing agro-wastes were adopted from [7] with few modifications. Apple pomace was dried in the shade and ground into fine particles and was sterilized by autoclaving at 121 °C and 15 psi for 15 min. After cooling 20 ml of seed culture of *S. diastaticus* was inoculated into the sterile apple pomace broth and incubated at 37 °C for 12 days. Growth of *S. diastaticus* in apple pomace and Starch Casein Broth (SCB), the control medium was monitored by measuring the optical density at 600 nm (OD 600 nm).

2.3 Preparation of cell free supernatant

After incubation, the fermentation broth was filtered and centrifuged at 5000 rpm for 15 min. The pellets were discarded and cell free supernatant was used for subsequent AgNPs synthesis.

2.4 Biosynthesis of AgNPs

AgNPs were biosynthesized using 5 mM AgNO₃ solution using cell-free supernatant to AgNO₃ volume ratio of 3:3. The reaction mixture was incubated at 37 °C for 24 h in the dark to facilitate nanoparticle synthesis. After 24 hours of incubation, the reaction mixture was observed for any visible colour change. The mixture was centrifuged for 15 min and the obtained pellets were washed three times with ethanol to remove impurities. The pellets were oven-dried at 40 °C until they were completely dried. The dried AgNPs were used for antibacterial activity and characterization. Cell free supernatant of *S. diastaticus* grown in SCB, was used as a positive control.

2.5 Characterization of AgNPs

UV-Visible spectroscopy (UV-Vis) was performed to determine the characteristic Surface Plasmon Resonance (SPR) of AgNPs. Fourier Transform Infrared (FTIR) spectroscopy of AgNPs was done to identify functional groups responsible for the reduction, formation and stabilization of AgNPs. The FTIR spectrum was recorded over a wave number range 4000-400 cm⁻¹. The crystalline nature of the synthesized AgNPs was characterized by X-ray diffraction (XRD). The obtained diffraction peaks were compared with standard Joint Committee on Powder Diffraction Standard (JCPDS card no: 04-0783) to verify crystalline phase of the AgNPs. Transmission Electron Microscopy (TEM) was done to determine the morphology, distribution and size of the synthesized AgNPs. The average particle size of the AgNPs was determined by using ImageJ software. A total of 50 nanoparticles were randomly selected and measured using a measurement tool in ImageJ and a scale bar.

2.6. Antibacterial Activity of the synthesized AgNPs

The antibacterial activity of the biosynthesized AgNPs was determined by agar well diffusion method on Mueller-Hinton Agar (MHA). Fresh 24-h-old cultures of the selected human test pathogens *Escherichia coli* (ATCC 4157), *Klebsiella pneumoniae* (ATCC 13882), and *Shigella flexneri* (ATCC 9199) were uniformly swabbed onto the surface of MHA plate. The synthesized AgNPs (20 mg) were dispersed in 1 mL of sterile normal saline. Wells of 6 mm were made on the MHA plates and different volumes 25, 50, 75, and 100 μ L of the dispersed AgNPs were dispensed into the wells. The plates were incubated at 37 °C for 24 h, after which the zones of inhibition were measured in millimetres (mm).

3. Results and Discussion

3.1 Biosynthesis of AgNPs

AgNPs were biosynthesized after 24 h of incubation in the dark. Contrary to most previously reported studies, in which AgNPs exhibited a colour change from yellow to brown (9,10), the AgNPs in the present study showed a colour change from reddish to dark olive-brown [11]. The observed dark olive-brown colour after incubation may be attributed to the interaction of silver ions with extracellular pigments and other bioactive metabolites produced by *S. diastaticus* in the fermentation broth. Figure 1 illustrates the colour

change from reddish to dark olive-brown following incubation, indicating the formation of AgNPs.



Fig 1: Biosynthesis of AgNPs using secondary metabolites of *S. diastaticus* (a) reddish appearance of supernatant of *S. diastaticus* before the addition of AgNO_3 solution (b) reaction mixture of cell-free supernatant and AgNO_3 solution showing a dark olive-brown colour after 24 h of incubation, indicating AgNPs formation.

Characterization of AgNPs

3.2 UV-Visible spectroscopy

The UV-Visible spectra of the synthesized AgNPs displayed characteristic surface plasmon resonance (SPR) peak at 410 nm (Fig. 2). The presence of this peak indicates possible reduction of Ag^+ to Ag^0 and suggests the formation of AgNPs. Previous studies have also reported peaks between 400-450 nm [12, 13].

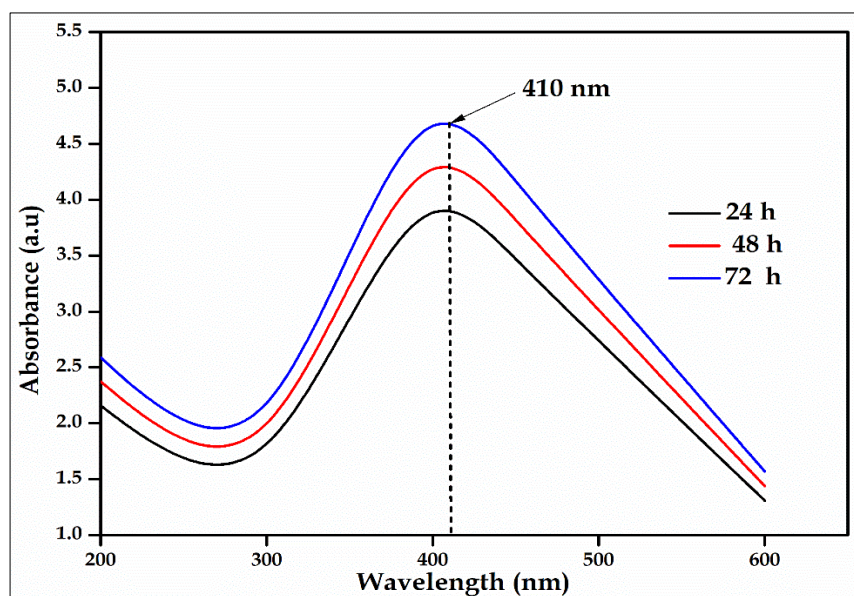


Fig 2: UV-Vis spectroscopy of the synthesized AgNPs from *S. diastaticus*.

3.3 FTIR analysis of the AgNPs

The FTIR spectrum (Fig 3) of the biosynthesized silver nanoparticles (AgNPs) revealed the presence of several functional groups associated with biomolecules involved in nanoparticle synthesis. The absorption band at 2980.59 cm^{-1} was assigned to C-H stretching vibrations, while the bands at 1559.95 cm^{-1} and 1067.69 cm^{-1} corresponded to cyclic alkene (C=C) and sulfoxide (S=O) functional groups,

respectively. These functional groups are likely associated with extracellular biomolecules that act as capping and stabilizing agents, thereby enhancing the stability of the AgNPs. The absorption band at 775.29 cm^{-1} was attributed to aromatic benzene, suggesting the presence of aromatic compounds that may have contributed to the reduction of Ag^+ ions during nanoparticle biosynthesis [14, 15].

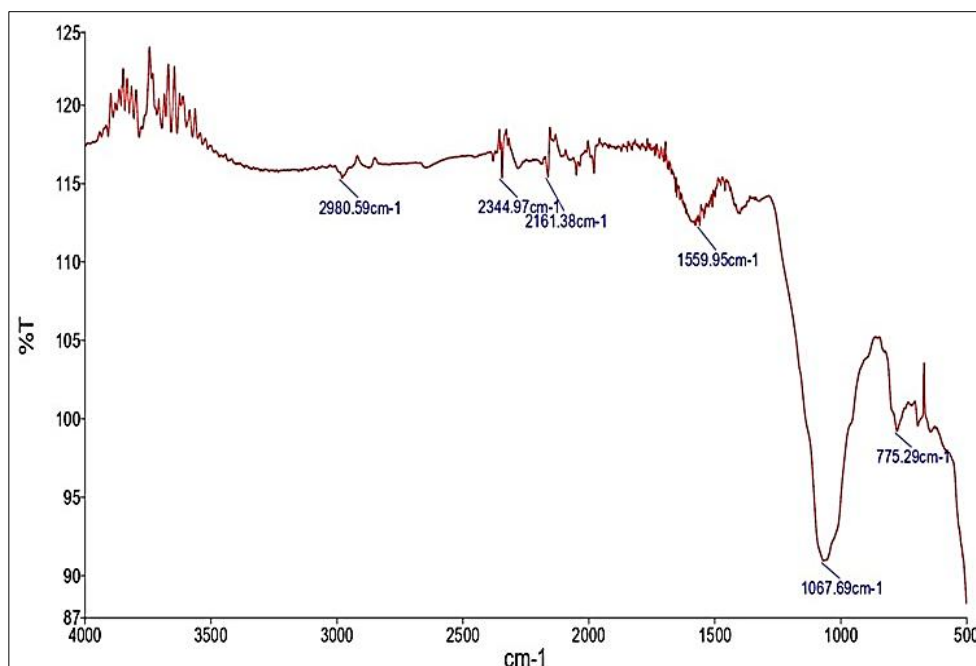


Fig 3: FTIR spectrum of the synthesized AgNPs synthesized by *S. diastaticus*.

Furthermore, the FTIR spectrum of the synthesized AgNPs indicates that the extracellular biomolecules produced by *S. diastaticus* during fermentation played a role as reducing and capping agents during nanoparticle formation. Apple pomace played a part in supporting the growth of *S. diastaticus* and the production of metabolites. The AgNPs were synthesized using the cell-free fermentation supernatant obtained after cultivating *S. diastaticus* in apple pomace broth. The cell-free supernatant was enriched with extracellular metabolites secreted by *S. diastaticus* during fermentation, indicating that these microbial metabolites were the primary reducing and capping agents responsible for the biosynthesis and stabilization of the AgNPs.

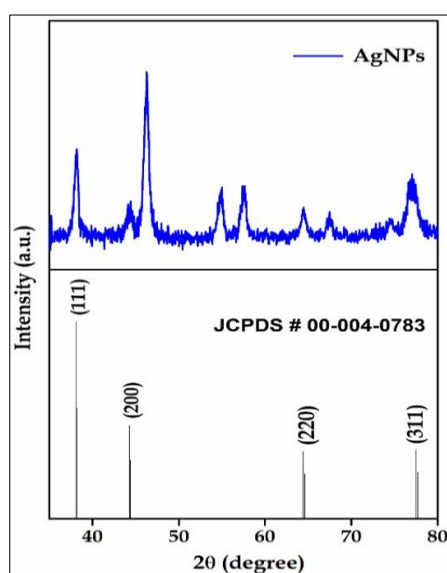


Fig 4: XRD analysis of the synthesized AgNPs from *S. diastaticus* in apple pomace

3.4 XRD analysis of AgNPs

The X-ray diffraction (XRD) pattern of the biosynthesized silver nanoparticles (AgNPs) is presented in Figure 4. Four characteristic diffraction peaks were observed at 2θ values of 38.22°, 44.55°, 64.45°, and 77.31°, corresponding to the (111), (200), (220), and (311) crystal planes of face-centred cubic silver (JCPDS Card No. 04-0783), thereby confirming the biosynthesis of crystalline AgNPs. The additional diffraction peaks observed in the XRD pattern may be attributed to residual biomolecules or bioactive metabolites produced by *S. diastaticus* that remained on the nanoparticle surface during biosynthesis. Similar diffraction patterns, with characteristic silver peaks and some unassigned peaks attributed to capping biomolecules, have been reported for *Streptomyces*-mediated AgNPs and other biologically synthesized AgNPs [16, 17].

3.5 Transmission Electron Microscopy (TEM)

TEM observations (Fig. 5) show that, the synthesized AgNPs from *S. diastaticus* exhibit a quasi-spherical shape. This structural characteristic has been reported in previous studies on AgNPs [18, 19]. The observed quasi-spherical form suggests minor deviations from the perfect spherical shape, which may likely have been due to differential nucleation and growth processes during nanoparticle formation. Furthermore, AgNPs synthesized by *S. diastaticus* showed uneven distribution with some areas of aggregation. Although FTIR analysis showed the presence of biomolecules which can act as capping and stabilizing agents, the observed aggregation suggests that, their capping efficiency was low, resulting in reduced colloidal stability. Furthermore, the average particle size of the synthesized AgNPs was 16.6 ± 4.49 nm. These findings are similar to the previous reported AgNPs [20].

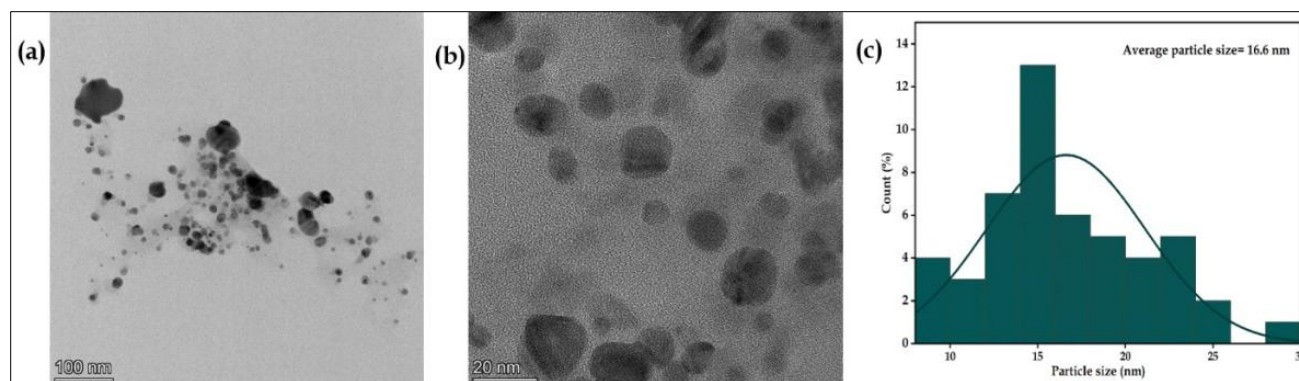


Fig 5: TEM micrographs of the synthesized AgNPs from *S. diastaticus* showing their morphology and distribution (a) 100 nm scale bar (b) High magnification TEM image at 20 nm scale bar, and (c) Average particle size distribution histogram

3.6 Antibacterial activity

The antibacterial activity of the biosynthesized silver nanoparticles (AgNPs) is presented in Table 1. The synthesized AgNPs exhibited antibacterial activity against all three tested human pathogens. The largest volume (100 μ L) produced the largest zones of inhibition, ranging from 14 to 16 mm, with the highest antibacterial activity observed against *Klebsiella pneumoniae* (16 mm). These findings are in close agreement with that reported in AgNPs study with zone of inhibition of 16.5 mm against *K. pneumoniae* [12]. The antibacterial activity of the biosynthesized AgNPs may be attributed to their ability to interact with the bacterial cell membrane, generate reactive oxygen species, release Ag^+ ions, and disrupt essential cellular processes causing bacterial cell death [21].

Table 1: Zone of inhibition of AgNPs synthesized from *S. diastaticus* against selected human pathogens

Test pathogens	Zone of inhibition (mm)			
	25 μ L	50 μ L	75 μ L	100 μ L
<i>E. coli</i>	0	8	12	14
<i>K. pneumoniae</i>	0	0	14	16
<i>S. flexneri</i>	8	11	13	15

Conclusion

The present study demonstrated the successful biosynthesis of silver nanoparticles (AgNPs) using *Streptomyces diastaticus* cultivated in apple pomace, highlighting the potential of agro-industrial waste as a sustainable substrate for AgNP production. The synthesized AgNPs were confirmed by a characteristic colour change and a surface plasmon resonance (SPR) peak at 410 nm obtained by UV-Visible spectroscopy. FTIR analysis revealed the presence of biomolecules involved in the reduction, capping, and stabilization of the nanoparticles, while XRD analysis confirmed their crystalline nature. TEM analysis demonstrated the formation of predominantly quasi-spherical nanoparticles with an average particle size of 16.6 ± 4.49 nm. The synthesized AgNPs exhibited antibacterial activity against *Escherichia coli*, *Klebsiella pneumoniae*, and *Shigella flexneri*, with the highest inhibition observed against *K. pneumoniae* at the highest tested concentration. These findings demonstrate that apple pomace can serve as an environmentally friendly substrate for the production of biologically synthesized AgNPs with promising antibacterial properties. Further studies should investigate the cytotoxicity, stability, and efficacy of the synthesized AgNPs against a broader range of multidrug-resistant pathogens to support their potential biomedical applications.

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