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Biosynthesis of silver nanoparticles from *Staphylococcus lentus* isolated from *Ocimum basilicum* and their antibacterial activity

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Abstract

In this century, the development of nanotechnology is projected to be the establishment of a technological evolutionary of this modern era. Recently, nanotechnology is of substantial research in modern material sciences and hence metal. The studies describing the biosynthesis of silver nanoparticles by bacteria followed by the investigation of synthesis mechanism and antibacterial activities may be useful for Nanobiotechnology research opening a new arena in this field. The present study was isolated and identification was present of endophytic bacterium isolated from *Ocimum basilicum* by the surface sterilization method. The bacterial strain was identified as *Staphylococcus lentus.* Bacterium isolate was used to detect their ability to prepare silver nanoparticles. The results showed a change in the color of the silver nitrate solution 1mM brown, studied the spectrum of absorption of UV-visible spectroscopy of the solution silver particles nanosecond step in making sure the formation of nanoparticles and was found to be located on the wavelength of 400 nm to bacteria. *Ocimum basilicum* also showed that the X-ray diffraction peak was at (103) and at the angle (34.1). Additionally, the results showed clear images in the scanning electron microscope size and spherical shape, Size ranged between 20.4 -93.04nm. The results revealed that AgNPs synthesized from *Staphylococcus lentus* have antibacterial activity against Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* with diameter inhibition zone 19.0 mm and 20.0 mm respectively.

Keywords: AgNPs, *antibacterial activity, SEM;Escherichia coli*, *Staphylococcus lentus*

Introduction

In the recent past, paramount importance is given to research in the field of nanotechnology owing to its applications in various fields (Banoon & Ghasemian, 2021). This led to the amalgamation of physical, chemical, biological and engineering sciences to develop innovative techniques in maneuvering and manipulating the matter at the atomic level. The emphasis on the materials at the nano levels is attracting attention because of the more pronounced properties exhibited by them at a small size. The certain phenomenon may be exhibited in a significant manner in the nanoscale rather than at the micro-level (Sunkar & Nachiyar, 2012). Endophytes were sheltered from environmental stresses and microbial competition by the host plant and appear to be ubiquitous in plant tissues, having been isolated from flowers, fruits, leaves, stems. Some endophytic bacteria exert several beneficial effects on host plants, such as stimulation of plant growth (Sturz et al., 1997). Use of a biological system like microbes for the synthesis of various nanoparticles has emerged as a novel research area (Li et al., 2011; Aldujaili & Banoon, 2020). Endophytic Microbes (Bacteria and Fungi) have been isolated from the interior of the stems and roots of many plants, such as ginseng, cotton, sweet corn, canola, wheat, and others (Cho et al., 2007; Hateet, 2020). Microbes that inhabit asymptomatically in the living tissues of plants without causing any substantive negative effect are known as endophytic microbes (Bacon & White, 2000; Rana et al., 2020), silver nanoparticles (Ag NPs) have shown excellent bactericidal properties against a wide range of microorganisms (Ahmad et al., 2020; Hassan et al., 2020). Biogenic synthesis of AgNPs involves bacteria, fungi, yeast, actinomycetes, and plant extracts (Siddiqi & Husen, 2016; El-Rafie et al., 2017; El-Ebissy et al., 2019). Recently, a number of parts of plants such as flowers, leaves and

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fruits, besides enzymes, have been used for the synthesis of silver nanoparticles (Husen & Siddiqi, 2014; Kuppusamy et al., 2016; Siddiqi et al., 2018). The size, morphology and stability of nanoparticles depend on the method of preparation, nature of the solvent, concentration, strength of reducing agent and temperature (Iravani et al., 2014). Antibiotic-resistant bacterial strains have been discovered from many environments, severely limiting therapeutic choices and endangering the lives of infected people (Banoon et al., 2020). Silver is used as an antibacterial agent since ancient times. Property of silver as the antibacterial agent has been used for bacterial infections, dental work, catheters, and burn wound treatment in different forms as silver nitrate, silver sulfadiazine and metallic silver, AgNPs have an important advantage over conventional antibiotics in that it kills all pathogenic microorganisms, and no organism has ever been reported to readily develop resistance to it (Dibrov et al., 2002; Abd Ali, & Shareef, 2021). The aim of this study is to isolate endophytic bacterium, characterize the synthesized AgNPs and study their antibacterial activity.

Materials and methods

Isolation and Identification of endophytic bacterium

Endophytic bacteria isolated refer (Linda et al., 2018). The sample leaf of *Ocimum basilicum* were done by surface sterilization (Hidayati et al., 2014). Each sample was cut with a size of 3 cm x 2 cm and then inoculated on a Petri dish containing Nutrient Agar (NA) medium and incubated at 37c for 72 hr. endophyte was grouped based on their phenotypic characteristics, for example, colony color and morphology, Gram reaction staining (Steinbach & Shetty, 2001). The morphology observation of one endophytic bacterium included bacteria colony color, colony shape, elevation and margin. Morphological observation on bacteria cell included cell shape and Gram staining (Hadioetomo, 1993).

Synthesis of nanoparticles

A hundred milliliters of sterile Luria Broth medium were prepared and inoculated with endophytic of bacterial suspension. The culture flasks were incubated for 36h at 37 °C with shaking at 150 rpm/min. After the incubation period, the bacterial cell pellet was collected by centrifugation at 10 000 r/min for 10 min. This biomass was washed thrice in sterile distilled water to remove any adhering nutrient media that might interact with the silver ions. About 1 g of the biomass was then suspended into 20 mL of 1 mM silver nitrate solution and incubated for 72-120 hr. at a temperature of 25°C (Sunkar & Nachiyar, 2012).

Characterization of synthesized silver nanoparticles UV- Vis spectroscopy

Silver nanoparticles synthesized by three methods were analyzed for UV-Vis spectroscopy. The UV-Vis spectroscopy measurements of silver nanoparticles were recorded on a Systronic double beamspectrophotometer:2202. Microbial synthesized silver nanoparticles were measured in a wavelength of 420 nm. Chemically synthesized silver nanoparticles were measured in a wavelength ranging from 200-1100 nm (Zhang et al., 2016).

Fourier transform infrared (FTIR) spectroscopy

The FTIR spectrum of the biosynthesized silver product was recorded on a FTIR instrument mode **Nicolet 6700** spectrometer at a resolution of 4 cm-1 attachment. All measurements were carried out in the range of 400-4000 cm^{-1}at a resolution of 4 cm^{-1} (Baudot et al., 2010; Zhang et al., 2016).

X-Ray Diffraction Analysis

The X-ray Diffraction (XRD) measurements of drop-coated films of Bragg angle 2θ at a scanning rate of 2° min^{-1}carried out on a Philips PW 1830 instrument that was operated at a voltage of 40 KV and a current of 30 mA with Cu Ka radiation (λ =1.5405 Å) (Waseda et al., 2011; Zhang et al., 2016).

Zeta-Potential measurements

The s-potential of the nanoparticles was measured with a Zeta sizer Nano ZS instrument (Malvern, UK).

Scanning Electron Microscopic (SEM) analysis

Analysis of the sample AgNPs was performed using SEM (INSPECT S50, FEI, Netherland). Thin films of the sample were prepared on carbon-coated copper grids by dropping an amount of the filtrate on the grid and the extra solution was removed by a blotting paper then the films on the grids were allowed to dry overnight at room temperature under a sterilized condition. SEM images of the silver nanoparticles were exposed at different magnifications (Fissan et al., 2014; Zhang et al., 2016).

Antibacterial Activity Test for AgNPs

The antibacterial effect of synthesized AgNPs was evaluated against some human pathogens such as gram-negative *E. coli* and gram-positive *Staphylococcus aureus* bacteria by disc diffusion method. Each strain was swabbed uniformly into the individual Muller-Hinton agar plates using sterile cotton swabs. Using sterile

micropipette, 30 µL of synthesized AgNPs were loaded on to sterile paper disc (0.6 mm) and it was allowed to dry. Plates were incubated at 37 °C for 24 hr., an appearance of inhibition zones around the filter paper disc indicating the bioactivity of synthesized AgNPs (Gould & Bowie, 1952). The diameters of the clear zones were measured and compared with Gentamycin (30 µL) (control); triplicates were made.

Results and discussion

Isolation of endophytic bacteria

From the surface-sterilized leaf segments of *Ocimum basilicum*, the endophytic bacteria started to grow from the cut ends after 48h and appreciable growth was observed after 72 h. The endophytic bacterium was identified as *Staphylococcus lentus*. Using the standard microbiological, biochemical tests and VITEK2 system according to Manufacturer's instructions (BioMerieux, 2010) Figure 1.

Synthesis and characterization of nanoparticle

The formation of nanoparticles is initially observed by color change from pale white to brown Figure 2. The characteristic brown color arises due to excitation of surface plasmon vibrations in the silver metal nanoparticles (Jha et al., 2009). we use UV-Vis spectroscopy to follow up with the reaction process. The spectra recorded from the reaction vessel after 72 hours reported in Figure 3. The time at which the aliquots were removed for measurement is indicated next to the respective curves. The strong surface plasmon resonance centered at ca. 400 nm, is characteristic of colloidal silver. This peak increased from 400 nm as the reaction proceeded. The spectra clearly showed the increase in the intensity of silver solution with time, indicating the formation of increased number of silver nanoparticles in the solution. According to the Figure 3, there is no appreciable change in the UV-Vis spectra of the reaction product after 72 hours indicative of the fact that the reaction came to equilibrium at about 72 hours.

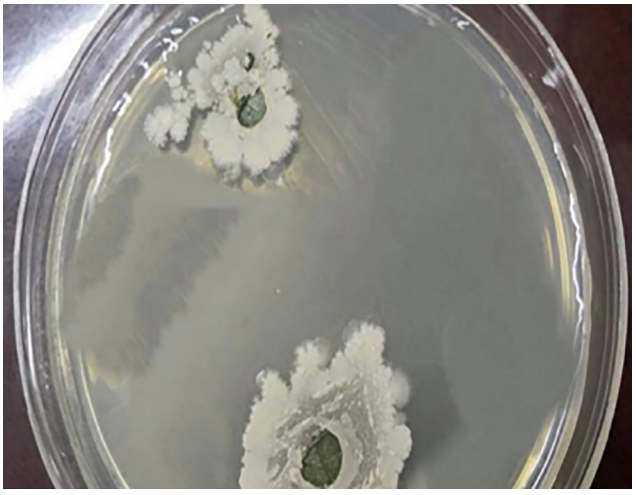

Figure 1. Endophytic bacterium from the surface-sterilized leaf segments of *Ocimum basilicum on nutrient agar.*

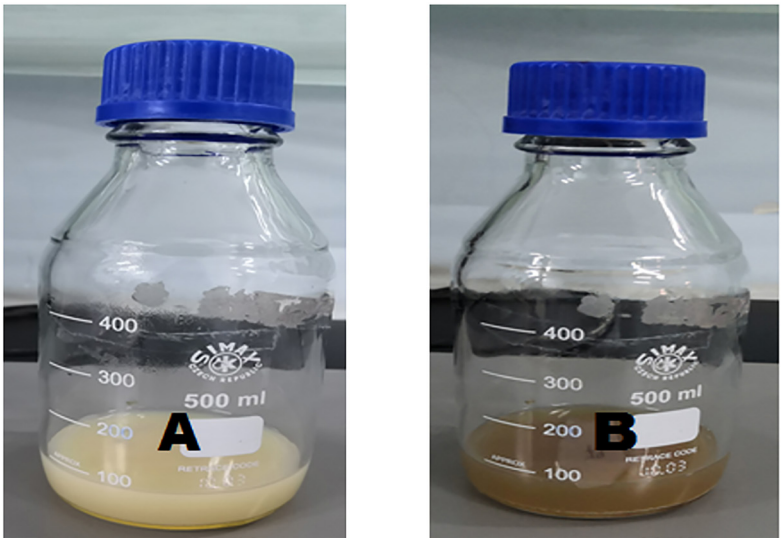

Figure 2. A) Filtrate of endophytic bacterium, *Staphylococcus lentus* . B) Color change to brown after treating with 1 mM $AgNO_3$.

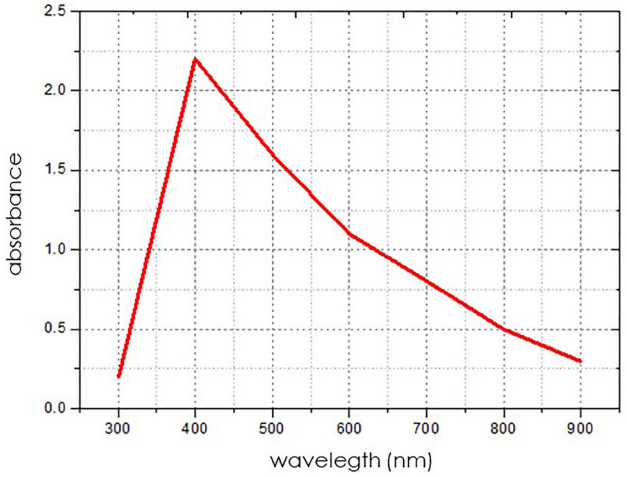

Figure 3. UV visible spectroscopy of $AgNO_3$ of endophytic bacterium, *Staphylococcus lentus*.

FTIR spectroscopy analysis

The FTIR spectroscopy analysis of AgNPs synthesized from the endophytic *staphylococcus lentus* bacterium showed the presence of a peak at 3417.75cm^{-1} refer to the bending vibration of the amide (N-H group) of protein while the band at 1629.34 cm^{-1} indicate the presence of (C=O-NH group and presence peak at 1385.10 cm^{-1} indicate the presence of (C-N group) Figure 4 Table 1, FTIR spectroscopy analysis very important to characterize the proteins binding with the AgNPs, and it is possible to quantify secondary structure in metal nanoparticle–protein interaction FTIR spectroscopy was carried out on AgNPs synthesized after 96 h of incubation with AgNO3.

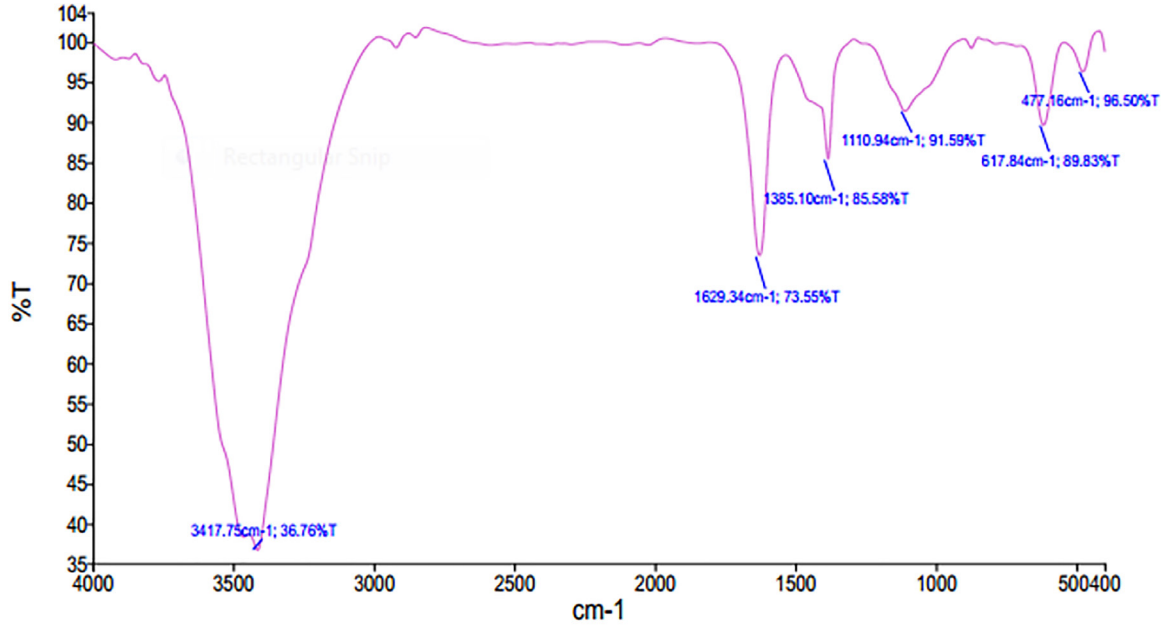

Figure 4. FTIR spectra of the silver nanoparticle suspension.

Table 1. FTIR spectra of the silver nanoparticle suspension showed the absorbency band of different chemical functional groups.

Functional groups	Treated AgNPs
N-H,OH	3417.75
C=O-NH	1629.34
C-N	1385.10
C-O	1110.94

Particle size analysis of AgNPs by dynamic light scattering

(DLS) system the result of DLS showed good results. The data of DLS supported that the Z-average (r.nm)was 43.20 and 0.279 PdI value. The obtained single peak indicated that the quality of the synthesized Figure 4a.

Zeta potential analysis

From Figure 4b it is evident that AgNPs exhibited narrow particle size distribution with a Z-average value of 75.39 nm and low polydispersity index (PDI) of 0.279. Zeta potential value -29.1 indicated that the capping molecules present on the surface of AgNPs are mainly comprised of negatively charged groups and are also responsible for moderate stability of the nanoparticles.

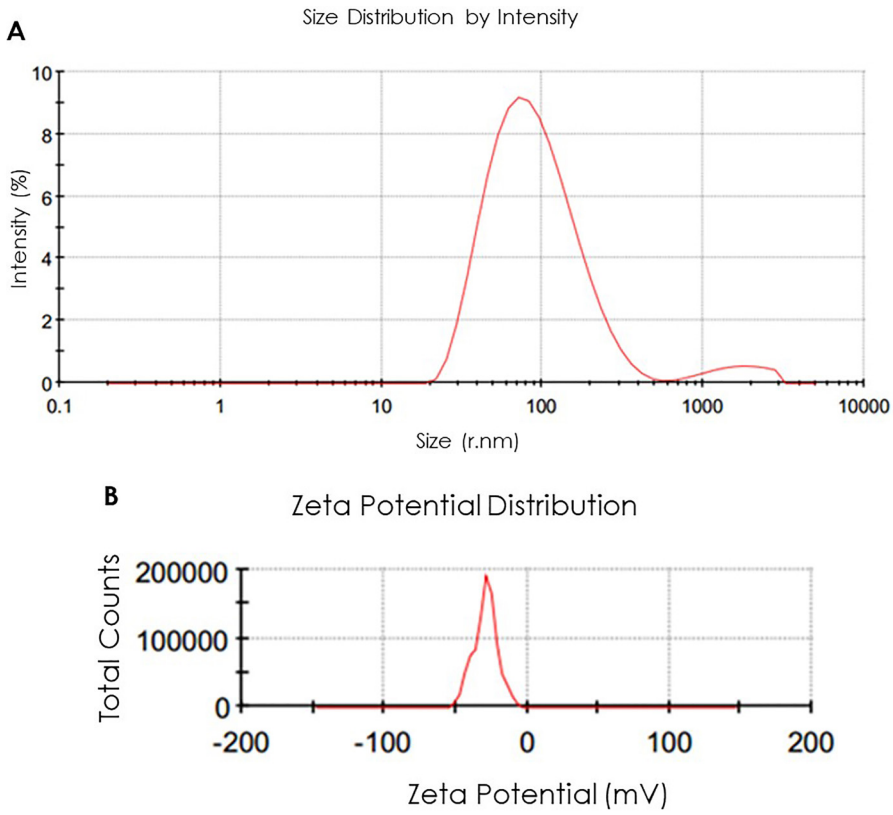

Figure 5. (a) DLS (b) Zeta potential graph of *S.lentus mediated* synthesized AgNPs.

X-Ray Diffraction Analysis

Further studies were carried out using X-ray diffraction to confirm the crystalline nature of silver nanoparticles. X-ray diffraction (XRD) is a popular analytical technique which has been used for the analysis of both molecular and crystal structures (Waseda et al., 2011). The evaluation of the XRD phase and crystal structure analysis of green synthesized AgNPs is shown in Figure 6. The reflections (103) of the spherical B structures of the AgNPs were calculated in the XRD analysis by 2θ (34.01o) values. It was determined that AgNPs have an elemental (Ago) and spherical crystalline structure.

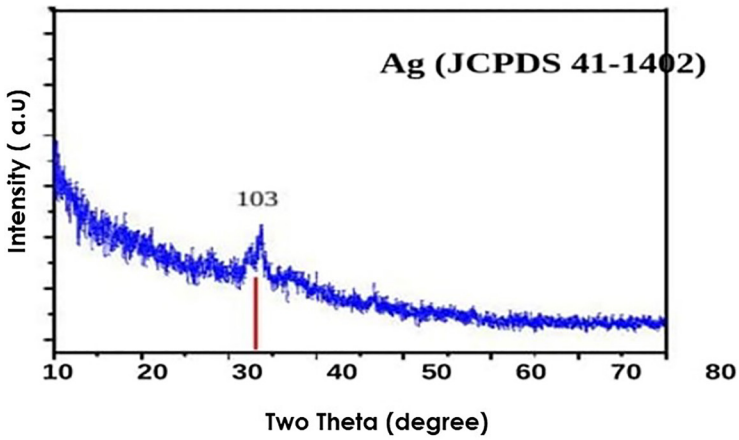

Figure 6. XRD of silver nanoparticles produced from an endophytic bacterium, *Staphylococcus lentus.*

Scanning electron microscopy

Morphological characteristics of AgNPs were investigated with a scanning electron microscopy (SEM). The results of Figure 7 demonstrated the presence of spherically shaped and size ranged between 20.4-93.04 nm. In general, the characteristics of monodispersed, shape, size of particles were highly dependent on the function of SNPs. The obtained data from SEM analysis were very similar to the findings of other researchers (Yousefzadi Nobakht & Shin, 2016).

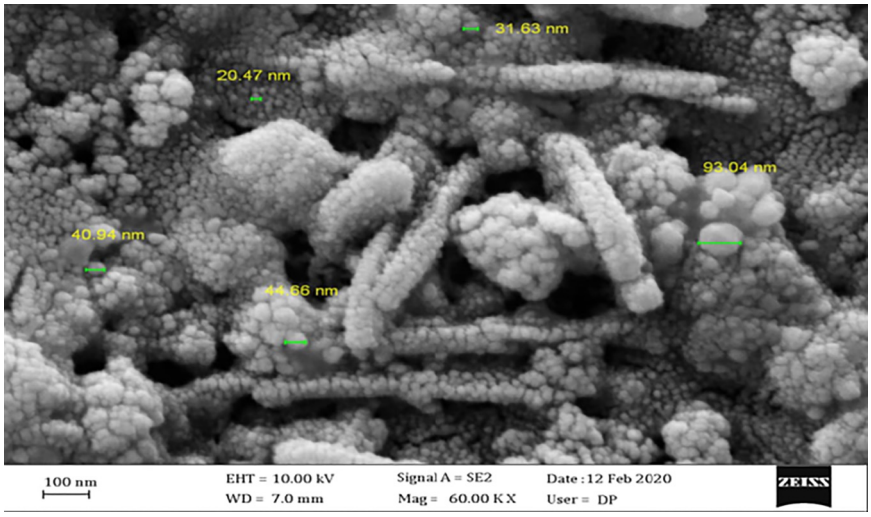

Figure 7. SEM Image show $AgNO_3$ synthesized by endophytic bacterium *S.lentus.*

Antibacterial assays of synthesized silver nanoparticles

In this study, the antibacterial effect of AgNPs obtained from *S.lentus* investigated by disc diffusion method. The possible antibacterial effects of AgNPs on Gram-negative *E. coli* and Gram-positive *S. aureus* strains and compare with Gentamycin. The antibacterial effect results of the produced AgNPs on *E. coli* and S. *aureus,* zone inhibition was found to be the range of (12.5-21.0) mm Table 2, Figure 8. Silver ions can interact with thiol groups in critical bacterial enzymes and proteins, and subsequently damage cellular respiration, resulting in cell death. The generation of ROS and free radicals (Kim et al., 2011). Antimicrobial activities of the secondary metabolite of endophytic fungus *S. radium* against the five bacterial pathogens was screened by paper disc diffusion method (Kim et al., 2011). The antibacterial effect was highly variable, ranging between 22.5-35.0 mm.

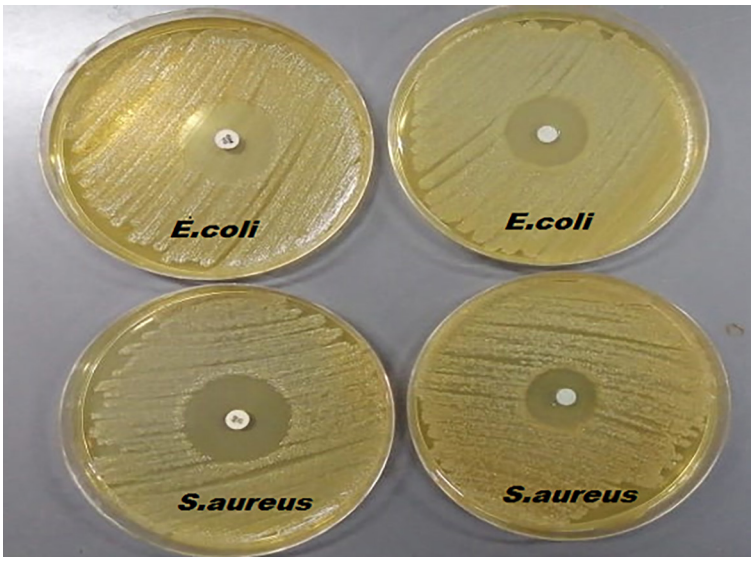

Figure 8. Inhibition zone created by AgNPs against *S.lentus*.

Table 2. Zones of inhibition produced by the biogenic silver nanoparticles against the pathogenic bacteria.

Test organisms	Zone of inhibition(mm) AgNps	Gentamycin
E.coli	19.0	20.0
S.aureus	21.0	12.5

Conclusions

The present study is an attempt for synthesis of silver nanoparticles from endophyte bacteria which was identified as *S.lentus*, the morphology and chemical composition of the silver nanoparticles were determined by different techniques such as UV-Vis, FTIR, XRD and SEM. These results suggest that the green synthesized Ag-NPs may serve as effective alternative antimicrobial agent's gram positive and gram-negative bacteria.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.