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Original Article

Biosynthesis of Silver Nanoparticles from *Streptomyces olivaceous* and its antimicrobial activity

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ABSTRACT

Nanotechnology has been considered as a description of activities at the level of atoms and molecules that have application in the real world. Nanoparticles are often in the range of 10-100 nm and this is the size of human proteins. Nanotechnology is widely used in the fields like medicines, food, consumer products energy, health care, environment, agriculture etc. Biological production using plant extracts and microbes are of special interest due to their effectiveness and flexibility. In this study, Silver nanoparticles were synthesized using *Streptomyces olivaceus sp* -1392, a member of actinomycetes. The produced silver nanoparticles were confirmed using Visible UV Spectrophotometer and characterized by FTIR and SEM analysis. The UV Spectrophotometer revealed the formation of Silver nanoparticles by yielding silver Plasmon absorption maxima at 420 nm and SEM micrograph indicates the uniform spherical particles at the size range of 500 nm to 1µm. The Fourier Transform Infrared Spectroscopy confirmed the presence of proteins as the stabilizing agent surrounding the Silver nanoparticle. The silver nanoparticle has the ability to inhibit the pathogenic organisms like *Staphylococcus aureus*, *E. coli* and *Bacillus cereus* and shows antibacterial activity.

Key words: Silver Nanoparticles, Streptomyces olivaceus, antibacterial activity

1. INTRODUCTION

Nanotechnology has been considered as a description of activities at the level of atoms and molecules that have application in the real world. A Nanometer is a billionth of a meter, is about 1/80,000 of the diameter of a human hair, or 10 times the diameter of a hydrogen atom. Nanoparticles are often in the range of 10-100 nm and this is the size of human proteins.

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Nanoparticles possess a very high surface to Volume ratio exhibit greater specific surface areas and surface energies, quantum related effects etc (Dagani, 2002). To compete with this tremendous demand, the synthesis of nanomaterials of specific composition, shape and size is a burgeoning area of research in the field of nanotechnology. Nanotechnology is widely used in the fields like medicines, food, consumer products energy, health care, environment, agriculture etc. Nanoparticles possess increased structural integrity as well as unique chemical, optical, mechanical, electronic and magnetic properties compared to large particles of bulk materials. The main approaches for fabrication of nanoparticles are top-down and bottom-up approach. The sizes of nanostructures produced by this method are between 10 to 100 nm. All physical, chemical and biological method used for synthesis of nanoparticle comes under these approach. "Green nanotechnology" is defined as the environmentally friendly manufacturing processes that reduce waste products which ultimately lead to atomically precise molecular manufacturing with zero waste. Green nanotechnology is of prime importance because chemical synthesis is toxic and leads to by products that are not environmental benign. In green nanotechnology the nanoparticles are synthesized by various plant extract and microbes. Silver is a highly stable metal which has a wide range of application from ancient days. A variety of techniques have been developed to synthesize metal nanoparticles, including chemical reduction using a number of chemical reductants including tri sodium citrate, ethanol, ethylene glycol and *N,N*-dimethylformamide[1], aerosol technique, electrochemical or sonochemical deposition photochemical reduction, and laser irradiation technique. [2] Silver nanoparticles are potent and broad-spectrum antibacterial agents with activity against diverse species within both Gram-positive and Gram-negative bacteria.[3] Silver nanoparticles are found to have wide applications in various areas like optical receptors, bio-labelling [4] sensors, [5] bio active materials [6, 7] signal enhancers in SERS based enzyme Immunoassay. [8] Silver nanoparticles are known for their antimicrobial activity. Biological production of Silver nanoparticles is of special interest due to their effectiveness and flexibility. Biological methods can be divided into two categories depending on the position where the nanoparticles (or) nanostructures are created as many micro organisms can provide inorganic materials either intra or extracellularly [9-11] and known to exhibit a wide range of biological activities. [12] Several plant species, microbes like bacteria, fungi, yeast are capable of producing silver nanoparticle both extracellularly and intracellularly.[13] Enzymes like Nitrate reductase and peroxidase helps in production of Silver nanoparticles in enzymatic process. The alternative method is Non-enzymatic reduction of silver ions by interaction with the cell wall functional groups and reduces silver ions to Silver nanoparticles. Several actinomycetes were found to synthesis Silver nanoparticles. The aim of this study is to bio synthesize silver nano particle from *Streptomyces olivaceus sp* -1392 and confirmation using UV Spectrophotometer. Characterization study was performed using SEM and FTIR. The antimicrobial activity of the produced Silver nanoparticles was checked against pathogens.

2. MATERIALS AND METHODOLOGY

2.1 Collection and preparation of streptomyces culture

Streptomyces olivaceus sp-1392 was obtained from Microbial Type Culture Collection, Chandigarh , India. The obtained species was inoculated in MGY media which acts as growth media for its growth and proliferation. *Streptomyces olivaceus sp-1392* was grown in Yeast malt broth at 37°C for 7 days. Then the

culture was grown in Starch casein broth with continuous shaking at 30°C for 9 days at 200 rpm. After incubation, the culture was centrifuged at 4000 RPM for 20 min. The culture and supernatant was collected separately and stored separately. The culture obtained was washed thrice using sterile distilled water. Then the culture was dissolved in sterile distilled water. This was used for inoculation.

2.2 Inoculation into various concentration of silver nitrate solution

The concentration of Silver Nitrate at which Silver nanoparticle produced was detected. For this, 0.1 M stock Silver Nitrate solution was prepared using sterile distilled water. The various concentration of Silver Nitrate solution from 0.5mM, 1.0mM, 1.5 mM, 2.00 mM and 2.5 mM were taken as 2 sets in 100 ml Erlenmeyer flask. To one set,5 ml of culture was added in each flask and to the other set, 5 ml of supernatant was added. This was kept for incubation for 20 days in dark condition. The UV spectrophotometer reading was taken at 400-450 nm. The concentration at which maximum absorbance got was observed. From this, the required concentration of Silver Nitrate for Silver nano particle production was detected. The silver nano particle production in the culture and supernatant was also checked by UV-VIS Spectroscopy and in which concentration Silver Nano particle synthesized was detected by change in color from white color to brown color. [14, 15]

2.3 Production of silver nano particle

The culture obtained by centrifugation was inoculated in 100 ml of 1 mM aqueous Silver Nitrate in 250 ml Erlenmeyer flask. In 100 ml of sterile Distilled water, the culture alone was inoculated. This acts as the control. Both were kept for incubation at 37°C for 20 days. After 20 days, the change in color from white to brown was observed. [16]

2.4 Detection of Silver nanoparticle

From the inoculated solution kept for incubation, 1ml of solution is taken aseptically and reading was taken in UV Spectrophotometer (Hitachi U 2900). The wavelength scanning was performed every 24 hours interval and the peak obtained was observed. The peak was got within the range of 300-700 nm.

2.5 Characterization of Silver Nanoparticle

FTIR analysis was carried out using Fourier Transform infrared (FTIR) Model: IR Affinity 1 Make: Shimadzu spectroscopy analysis. All measurement was carried out in the range of 500 – 3500cm^{-1} at a resolution of 4 cm^{-1}. Scanning electron microscope (Jeol 6390;Hitachi;Japan) was used to obtain the surface image and the size of the microbially synthesized silver nanoparticle. [17]

2.6 Antimicrobial activity of Silver nanoparticle produced by Streptomyces olivaceus

Test organism used for the studies were obtained from MTCC. The organisms used were *E. coli*, *Staphylococcus aureus, Klebsiella pneumonia* and *Bacillus cereus.* The various test organisms were swabbed in individual Muller Hinton plates. Using 20µl sterile micropipette 20µl of the compound solution was added to one of the well, Ethyl acetate was added to other well and Streptomycin (30 mcg) was kept in other part. These plates were incubated at 37°C for 24 hours. After incubation, the different levels of zone of inhibition were measured using the Hi media antibiotic zone scale.

3. RESULTS AND DISCUSSION

3.1 Optimization for silver nanoparticles production

Addition of various concentration of $AgNO_3$ to the *S. olivaceous* pellet and supernatant led to change in color at 1mM concentration of $AgNO_3$ which was added to the pellet. This shows that 1mM is the optimum concentration required for Silver Nanoparticles production. There was no color change observed in supernatant when $AgNO_3$ was added to it. This shows

that the pellet has the ability to convert $AgNO_3$ to Silver Nanoparticles. This was primarily observed by taking reading in UV-spectrophotometer at 420 nm in 1mM concentration. From this it was confirmed that Silver Nanoparticles was synthesized intracellularly by *S. olivaceous.* The UV Spec reading is shown in Table 3:1 and the color change and Absorption spectra at 1mM concentration is given Fig 3.1, 3.2.

3.2 Mass production of silver nanoparticles from streptomyces olivaceus sp- 1392

On addition of *Streptomyces olivaceus* biomass to 1mM Silver nitrate solution and incubated for 25 days the change in color was observed. The color changes from colorless to yellowish brown color. The control (without Silver nitrate ions) showed no change of color when incubated in the same condition. The appearance of a yellowish brown color in the solution containing the biomass was a clear indication of the formation of Silver Nanoparticles nanoparticles in the reaction mixture. [18] The flask was almost colorless before the addition of Silver nitrate and the color changes to yellowish brown color on the completion of the reaction. Thus, it was evident that the cultures produce enzymes that reduce Silver nitrate to Silver ions. This reduction clearly indicates that the reduction of ions occurs through intracellular method. The silver nanoparticle exhibit striking color light yellow to brown, due to excitation of surface Plasmon vibration in the particles and thus provide a convenient means of visually determining their presence in the reaction which was detected using UV Spectrophotometer.[19] The figure 3.3 represents the color change from colorless to yellowish brown

3.3 UV spectrophotometer analysis

The primary detection for synthesis of nanoparticle is done by UV Spectroscopy. A surface Plasmon peak located at interval of 400-420 nm was observed for the Silver Nanoparticles production from *Streptomyces olivaceus.* It is found that the ultraviolet detection of Silver Nanoparticles consists of a few humps. These humps show than the molecule is absorbing radiation over a band of wavelengths. One reason for this band was observed because of electronic level transition which was usually accompanied by a simultaneous change between the more numerous vibrational levels. (Figure 3.4) shows the absorption spectra of Silver nanoparticle produced by *Streptomyces olivaceus.*

3.4 FTIR Analysis

The silver nanoparticles synthesized using *Streptomyces olivaceus* showed strong bands at 3427.51, 1010.70 cm^{-1} corresponds to stretch in OH band (3200-3600). The level of C=O ranges from 1647.21 to 1739.79 cm^{-1}. The peaks which was obtained 3736.12, 3427.51 and 3412.08 shows the presence of absorbance at amine (N-H) region. The peak 2974.23 represents stretch at C-H. The peak at 1641.42 shows presence of –C=C- region. The peak value obtained for C-N region is 1286.52. The occurrence of a spectrum over wavelength range of 1200- 1000 cm^{-1} indicates the presence of the polysaccharide in the synthesized nanoparticles. This is shown in Fig 3.5

3.5 Scanning electron microscope analysis

From Figure 3.6, Scanning electron micrograph confirms data obtained from the Laser diffraction. The size of 1µm was obtained at 10,000 magnification. From figure 3.7, the size of Silver Nanoparticles nanoparticle was found to be 5µm. when observed at the magnification of 4,000.

3.6 Anti microbial activity of silver nanoparticle

Silver nanoparticle exhibit antibiotic properties against various pathogens. The antibacterial activity of Silver Nanoparticles particle produced by *Streptomyces olivaceus* was studied against various Gram positive and Gram negative strains like *Staphylococcus aureus, E.coli, Klebsiella pneumonia* and *Bacillus cereus* were

used. The anti bacterial activity was determined by well diffusion method. The activity of silver nitrate, Silver Nanoparticle and *Streptomyces olivaceus* culture were studied. Figure 3.8 shows the Zone of inhibition against Gram positive and Gram negative pathogens *Staphylococcus aureus, E. coli, Klebsiella pneumonia* and *Bacillus cereus*.

The antibiotic disc Streptomycin 10 mcg was used as positive control and distilled water of 20µl was used as negative control. The Silver nitrate, Silver Nanoparticles and culture of 20µl was added separately to the plates and the activity was observed. Table 2 represents the Zone of inhibition for the pathogenic organism. For Silver Nanoparticles, the highest inhibition was found in *Klebsiella pneumonia* followed by *E.coli* and *Staphylococcus aureus.* There was no zone of inhibition observed in *Bacillus cereus.* There was no inhibition found in culture, which proves that the culture has no antibacterial activity. The zone of inhibition for Silver nitrate was high in *E.coli* followed by *Bacillus cereus* and *Staphyloccus aureus*. The silver nanoparticles showed higher zone of clearance compare to silver nitrate.

4. CONCLUSION

In this study the optimum concentration of Silver Nitrate for Silver Nanoparticles production, synthesis, antibacterial and anticancerous activities have been examined. The optimum concentration of 1mM inoculated in *Streptomyces olivaceus sp-1392* biomass was used for Silver Nanoparticles production. The characterization of silver ion exposed to the biomass and the reduction of silver nitrate was detected by UV Spectrophotometer. The various types of bonds and stretches present in the Silver Nanoparticles has been studied using Fourier Transform Infrared Spectroscopy. The proteins present around also detected using FTIR. The size of the Silver Nanoparticles has been detected by SEM analysis. The reduction of Silver nitrate to Silver Nanoparticles was done by intracellularly. The Silver Nanoparticles obtained have shown bactericidal effect against some pathogens. The future study is to determine the anti cancerous activity of the produced Silver nanoparticle will be analysed.

Table 1: UV Spectrophotometer reading for various concentration of $AgNO_3$ in pellet and Supernatant of *S. olivaceus*.

Concentration of $AgNO_3$	OD for Pellet	OD for Supernatant
0.5 mM	376	340
1 mM	420	356
1.5 mM	410	367
2 mM	396	378
2.5 mM	370	389

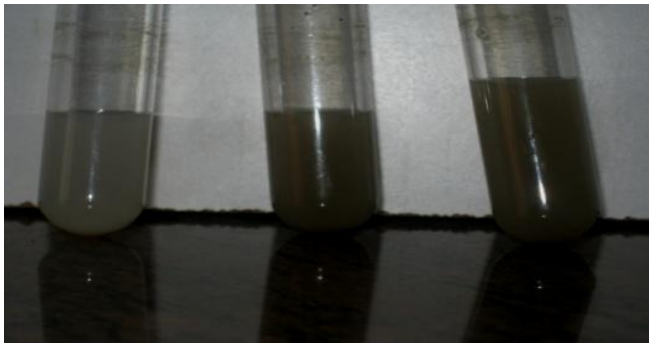


Fig 3.1: Color change in Pellet in various concentration of $AgNO_3$

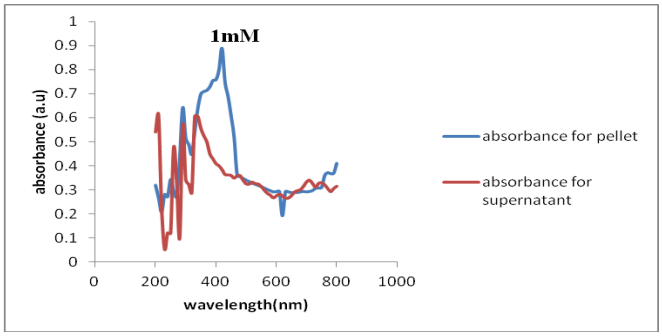


Fig 3.2: The absorption spectra for various concentration of $AgNO_3$ in which 1mM shows highest absorption at 420 nm

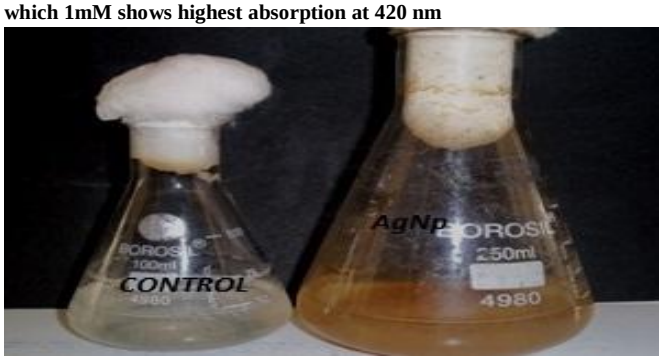


Fig 3.3: Production of Silver Nanoparticles from *Streptomyces olivaceus* Silver Nanoparticles- Silver Nanoparticle

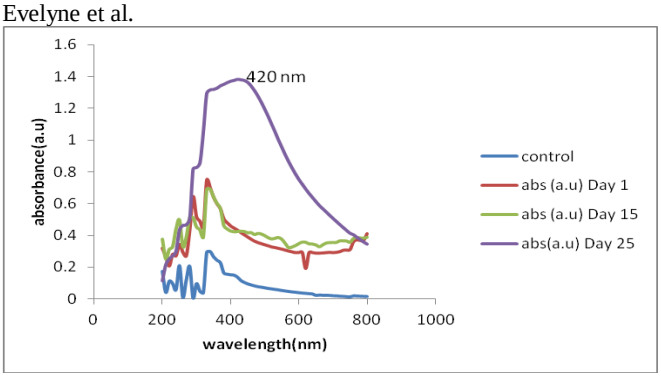


Fig 3.4: The absorption spectrum of Silver nanoparticles synthesized by *Streptomyces olivaceus*

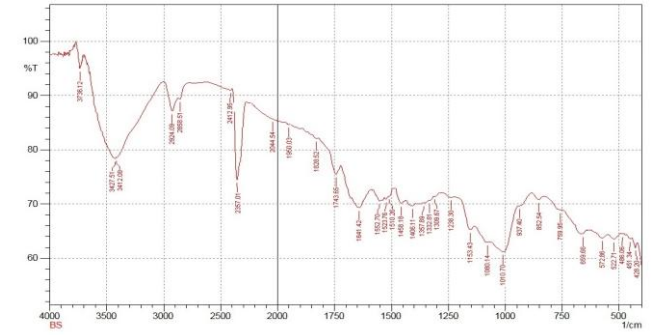


Fig 3.5: FTIR analysis of Silver nanoparticles produced by *Streptomyces olivaceus*

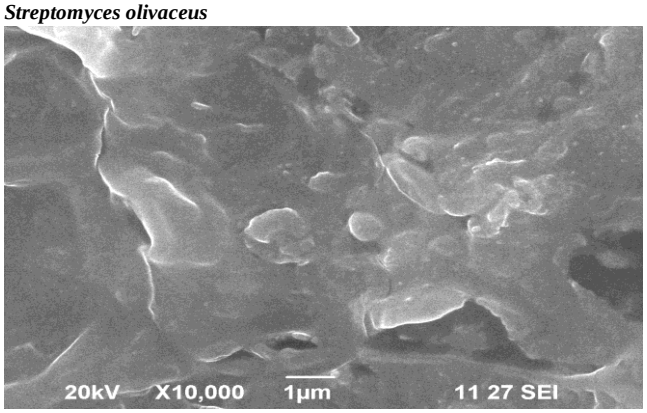


Fig 3.6:SEM analysis of biologically synthesized Silver nanoparticle at 10,000 magnification

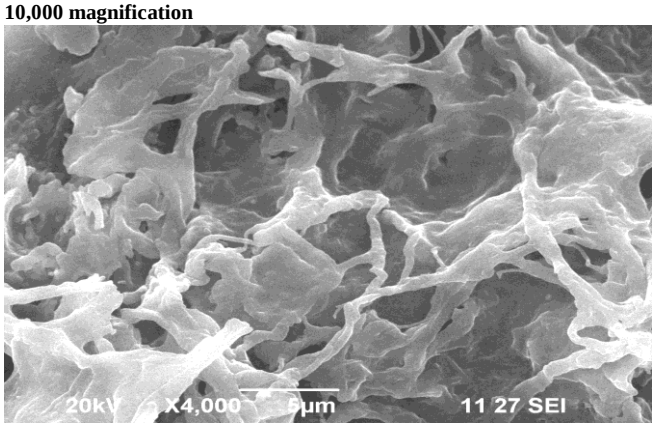
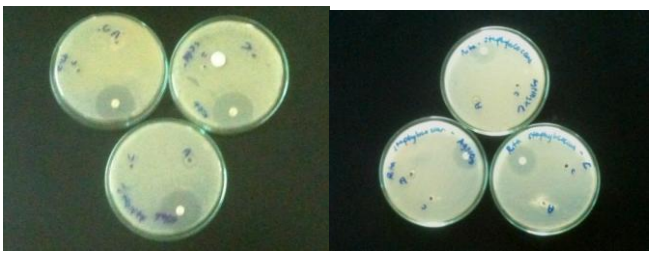


Fig 3.7: SEM analysis for Silver Nanoparticle at 4,000 magnification

Bacillus cereus ***E.coli***



Staphylococcus aureus ***Klebsiella pneumonia***

Fig 3.8: Antibacterial activity of Silver nitrate, Silver nanoparticle and culture against pathogens

Table 2: Zone of inhibition for Silver Nitrate, Silver Nanoparticle and *Streptomyces* culture

Culture	Antibiotic Disc(mm)	Control (mm)	$AgNO_3$ (mm)	Silver Nanoparticles (mm)	*Streptomyces olivaceus*(mm)
Bacilllus cereus	2.8	-	1		-
Klebsiella pneumonia	3	-	-	1.5	-
E.coli	2.9	-	1	1.2	-
Staphylococcus aureus	2.6	-	1	1.3	-

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