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Biosynthesis of silver nanoparticles using *Streptomyces griseus* PDS1 for anticancer activity

M.Suba Sri, M.Mekala, K.Suganya And B.Nandhini
Department of Microbiology, Sri Ramakrishna college of Arts and Science for women, Coimbatore, Tamilnadu,India.

Abstract: *Biological synthesis of nanoparticles provides advancement over chemical and physical methods. The use of micro-organisms to synthesize functional nanoparticle has been of great innovative interest. The research work focus on synthesis of silver nanoparticle using Streptomyces griseus PDS1. Both crude and filtered culture developed into nanoparticle. The reduction of silver ions was observed and monitored with the help of UV spectrum. Further an array of absorbance bands of silver nanoparticle was observed in FTIR. The size of the nanoparticle was observed under scanning electron microscope. The antimicrobial activity of produced nanoparticle was performed against the bacteria such as Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli and fungi Aspergillus niger. The development of cotton gauze with nanoparticle against Pseudomonas aeruginosa for anti cancer activity was recorded for biomedical application.*

Keywords: *Silver nanoparticle, Streptomyces griseus, antimicrobial activity, anticancer activity.*

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I. Introduction

The Silver nanoparticles are being used in numerous fields and incorporated into a wide range of desirable products. On comparison with biological synthesis and physio-chemical method of nanoparticle production leads to the presence of some toxic chemical substances adsorbed on the surface of skin may have adverse effect. Bioinspired synthesis of nanoparticles provide an advancement over chemical and physical methods (Raid Salih Jawaad *et al.,* 2014). So the nanoparticles are synthesized using micro-organisms with the metals like silver and gold which provides an eco-friendly process. In this Present work the silver nanoparticle is biologically synthesized using Actinomycetes- *Streptomyces griseus* PDS1 HM598125.

Streptomyces griseus is a species of bacteria in the genus Streptomyces which commonly found in soil. It is a gram positive bacterium with high G+C content (72.2%). They form a complex substrate mycelium that aids in scavenging organic compounds from their substrates (Keith *et al.,* .1984). More than 23 thousand bioactive microbial products including eight thousand anti-infective were demonstrated the increasing relevance, so called actinomycetes are sources of antibiotic (Lazzarini *et al.,* 2000). Some of the antibiotics produced by *streptomycetes* species are streptomycin, neomycin, chloramphenicol, and tetracycline. *Streptomycetes* species are resistant to its own antibiotic streptothricin but it is sensitive to streptomycin and actinomycin. The silver nanoparticles were synthesized using *Streptomyces griseus*. Characterization of nanoparticle was determined by UV-Visible nano drop spectrophotometer by the formation of plasmon peak. The chemical bonds present in the produced nanoparticle were confirmed by Fourier transform infra red spectroscopy. The size of nanoparticle was determined by scanning electron microscope. The developed nanoparticle was subjected to antimicrobial activity by well diffusion method. The development of cotton gauze with nanoparticle against *Pseudomonas aeruginosa* was recorded for biomedical application. The anticancer activity of the produced nanoparticle was done by MTT assay and the results were recorded by UV spectrophotometer.

II. Materials And Methods

2.1 Culture maintenance and synthesis of silver Nanoparticles

The bacterial culture of *Streptomyces griseus* PDS1 NCBI HM598125 was obtained from Centre for Bioscience and Nanoscience Research, Eachanari, Coimbatore. The culture was grown in Actinomycetes isolation agar at room temperature for 5 -7 days. The grown cultures were utilized for further purpose. For the synthesis of silver Nanoparticles, the Streptomyces culture was grown in 100 ml conical flask containing 25 ml of actinomycete broth and the culture was grown with Shaking Incubator at 28℃ for 4 days (120 rpm). The 5ml of entire culture from the broth is suspended into Eppendorf's tube and centrifuged at 5000rpm for 5 to 10 minutes, and then the supernatant was collected. To the 5 ml of Supernatant 2.5ml of 1mM $AgNO_3$ was added

and kept in a dark place without disturbance for 24 to 48 hrs. Similarly, to the 2ml of entire culture 1.5ml of 1mM $AgNO_3$ is added and kept in a dark place without disturbance for 24hrs.

2.2 Characterization of Silver Nanoparticles

2.2.1 UV-Visible Nano drop spectrophotometer

The reduction of silver ions was determined by measuring the UV-VIS spectrum of the reaction medium at 24 hrs time interval by drawing $1cm^3$ of the samples and their absorbance was recorded at a resolution at 300-600nm using UV-VIS spectrophotometer – (Elico, UV-VIS SL 159). Formation of Plasmon peak was observed and recorded.

2.2.2 Fourier Transform Infra-red Spectroscopy:

The chemical bonds present in the analyzed chemicals can be interpreted by FTIR spectrum by using the KBr pellets with prominent Resonance Spectra. The filtrate containing the extra cellular proteins secreted by the bacteria in the presence of Ag was salted out overnight at 40˚C using ammonium sulphate precipitate followed by centrifugation at 5000 rpm for 10 minutes. The protein obtained thereafter was dissolved in the minimal volume of deionized water and dialysed using a 12 KDa cut off dialysis membrane.

2.2.3 SEM (Scanning Electron Microscope)

Scanning Electron Microscopic (SEM) analysis was done at Cochin University, Kerala. Thin films of the sample was prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid was allowed to dry by putting it under a mercury lamp for 5 minutes for emitting characteristic X-rays. These characteristic X-rays are used to identify the composition and measure the abundance of elements in the sample.

2.3 Antimicrobial Activity:

2.3.1 Antibacterial activity of Silver Nanoparticle:

The antibacterial susceptibility test were carried out by using *Streptomyces griseus PDS1* silver nanoparticle against the micro organisms like *Escherichia coli, Klebsiella pneumonia, Bacillus subtilis* and *Pseudomonas aeruginosa* using well diffusion method. The cultures were swabbed and wells of 4mm size were made using sterile cork-borer in the nutrient agar; the wells were loaded with the 10µl of nanoparticles, and 30mcg of methicillin and 10µl of 1mM $AgNO_3$ which was used as control. The plates were incubated at 37 ºC. The zone of inhibition was observed and recorded.

2.3.2 Antifungal activity of silver nanoparticle:

The antifungal susceptibility test was carried out by using *Streptomyces griseus PDS1* Silver nanoparticles against the fungi like *Aspergillus niger* using well diffusion method. The cultures were swabbed onto the sterile malt agar medium and the wells of 4mm size was created using sterile cork-borer, the wells were filled with 10µl nanoparticles and 10µl of 1mM $AgNO_3$ which was used as control. The zone of inhibition was observed and recorded.

2.4 Development of cotton gauze using *Streptomyces griseus* nanoparticle:

Antimicrobial activity was determined using *Pseudomonas aeruginosa* by the development of cotton gauze cloth using *Streptomyces griseus* PDS1 Silver nanoparticle. Three gauze cloths were prepared, sterilized and kept in a nutrient agar plate. To that 10µl of synthesized silver nanoparticle, 10µl of supernatant and 10µl of Silver nitrate (Control) were added on it and incubated at 37℃/24 hrs. The zone of inhibition was recorded.

2.5 Anti-cancer activity:

Cell viability and Cytotoxicity assays were performed to find the anti cancer activity of the produced nanoparticle. The Dulbecos Modified Eagle Medium(DMEM) 0.195g, Glucose 0.045g, Sodium carbonate 0.037g was prepared in 10 ml T Flask. To this medium HeLa cell lines ($1X10^4$ cells) were inoculated. The media was incubated at 37˚C/24 hrs. The test dilution 500 µl and 250 µl were added along with Cell line 500 µl and MTT dye 500 µl. MTT assay were performed in which MTT (3-(4,5-dimethylthiazol-2-y)-2,5-diphenyltetrazolium bromide) is reduced to purple or dark-blue formazan in viable cell.

III. Results And Discussion

3.1 Physical Observation

The development of Silver Nanoparticle in the solution of 1mM $AgNO_3$ and sample of *Streptomyces griseus* PDS1 was confirmed by change in the color to pale yellow in Figure 3. Similar results were recorded by Saminathan. 2015.

3.2 Characterization of Silver Nano particle:

3.2.1 UV-Vis Nano drop spectrophotometer

The graph 1 determines the synthesis of silver nanoparticle using culture filtrate. The graph 2 determines the synthesis of silver nanoparticle using the entire culture of *Streptomyces griseus.* The Culture filtrate with plasmon peak obtained at 490 nm confirms the presence of Nano particle. Similarly the Culture with plasmon peak was obtained at 360 nm confirms the presence of nanoparticle. Dattusingh *et al.,* 2014 reported that the UV Vis spectrum of AgNps showed characteristic surface plasmon absorption band at the range of 483 nm. Saminathan. 2015 reported that the strong characteristic absorbance peak at around 450 nm was observed in the silver nanoparticle. In the UV absorption spectrum a strong broad peak was observed at 420 nm and 430 nm (Jeevan., 2011).

3.2.2 Fourier Transform Infra-red Spectroscopy:

FTIR Spectral analysis showed array of absorbance bands in 400 cm^{-1}- 4000 cm^{-1} were represented in the graph 3. The Spectral bands were prominent at 3368.27 cm^{-1} were assigned to the stretching vibrations of primary and secondary amines, the peak at 1856.45 cm^{-1} is a anhydride (C=O stretch). The bands seen at 1387.46 cm^{-1} corresponds to –C-N stretching vibrations. While the band at 524.12 cm^{-1} corresponds to (C-Br Stretch) Halogen. Similar results were recorded in Dattu Singh *et al.,* 2014 and Jeevan., 2011).

3.2.3 SEM (Scanning Electron Microscope)

The SEM images at 500 magnifications obtained and revealed the presence of silver nanoparticles that are aggregated. In the micrographs it has been observed that the nanoparticles were in the size ranging from 23 ± 78nm with a variety of morphology in structure (Figure 1). Similar results were recorded by (Saminathan. 2015).

3.3 Antimicrobial activity

3.3.1 Bacterial susceptibility of Silver nanoparticle:

The antibacterial activity of Silver nitrate the Silver Nanoparticles produced by *Streptomyces griseus* PDS1shows higher zone of inhibition against the test pathogens *Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus subtilis* and *Escherichia coli.* Compared to the antibacterial activity of antibiotic methicillin the silver nanoparticles produced by *Streptomyces griseus* PDS1 shows higher zone of inhibition against the test pathogens *Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus subtilis* and *Escherichia coli* respectively in Figure 2 and zone of inhibition are tabulated in Table1. Similar results were recorded for Antibacterial activity of silver nanoparticles against *Escherichia coli* (Zhao *et al.,* 1998).

3.3.2 Fungal susceptibility of Silver nanoparticle:

The antifungal activity of silver nitrate the silver nanoparticles produced by *Streptomyces griseus* PDS1 shows higher zone of inhibition against the test pathogen *Aspergillus niger* respectively. Compared to the antifungal activity of antibiotic methicillin the silver nanoparticles produced by *Streptomyces griseus* PDS1 shows higher zone of inhibition against the test pathogen *Aspergillus niger* respectively in Figure 3 and zone of inhibition are tabulated in Table 2.

3.4 Development of cotton gauze using *Streptomyces griseus* nanoparticle:

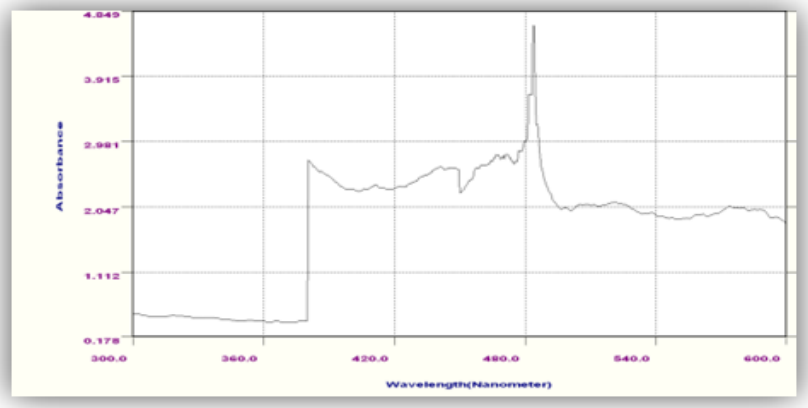
The biomedical application was carried out for the produced nanoparticle using culture filtrate of *Streptomyces griseus* againt a wound causing pathogen *Pseudomonas aeruginosa* with gauze cloth in Figure 4 and the zone of inhibition were tabulated in Table 3.The silver nanoparticles synthesized using sample of *Streptomyces griseus* PDS1 showed higher zone of inhibition than silver nitrate. The silver nanoparticles synthesized using sample of *Streptomyces griseus* PDS1 can be used for medical textile coating. Similar results were observed by Nithya *et al.,* 2009. As streptomyces were developed as nanoparticle and compared with commercial drugs such as Ampicillin and Tetracycline. The similar work were reported on the development of Nanoparticles and Nanoecapsulation with Ampicllin (Mekala *et al.,* 2016) and development of Nanoparticles with Tetracycline with Interfacial deposition of PHB (Mekala *et al.,* 2016).

3.5 Anticancer activity:

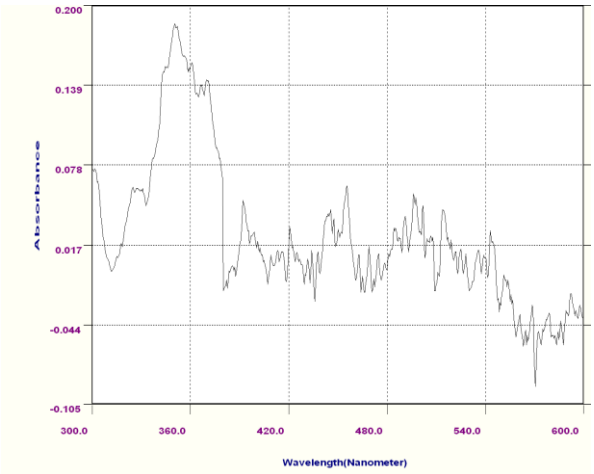
The cell viability was determined by MTT assay. Hela cell line were inoculated and incubated. The colour change from pink to yellow was observed. The MTT reagent yields low background absorbance values in the absence of cells. For each cell type the linear relationship between cell number and signal produced were established, thus allowing an accurate quantification of changes in the rate of cell proliferation. The values were tabulated in Table4.

IV. Conclusion

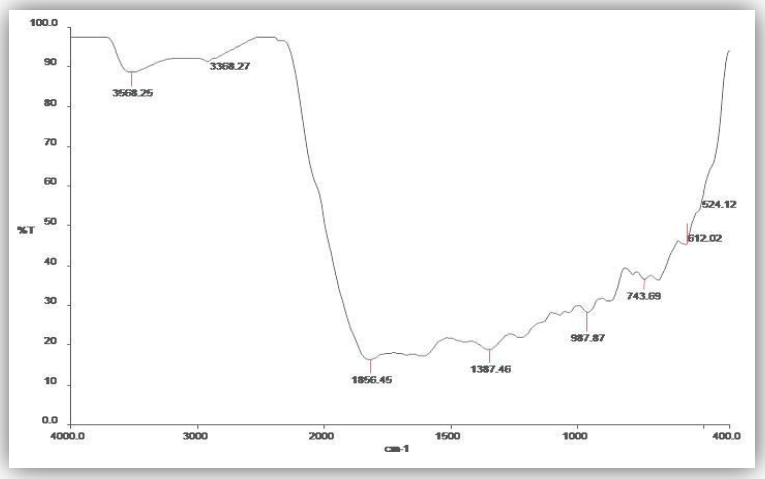
The Nanoparticle synthesized was confirmed by UV Vis Nano drop Spectrometer and FTIR and the size of the Nano particle was determined by SEM analysis. The Anti-microbial activity of silver Nano particle (Culture filtrate) has bactericidal and fungicidal effect than the Silver nitrate (Control). The *Pseudomonas aeruginosa* a wound causing pathogen gets suppressed on coating the medical textile with the sample. The sample has cytotoxicity activity against HeLa cell line was observed.



Graph1: *Streptomyces griseus* PDS1 Culture filtrate in UV- Vis Spectroscopy



Graph 2: *Streptomyces griseus* PDS1 Culture in UV-Vis Spectroscopy



Graph 3: *Streptomyces griseus* PDS1 Culture filtrate in FTIR

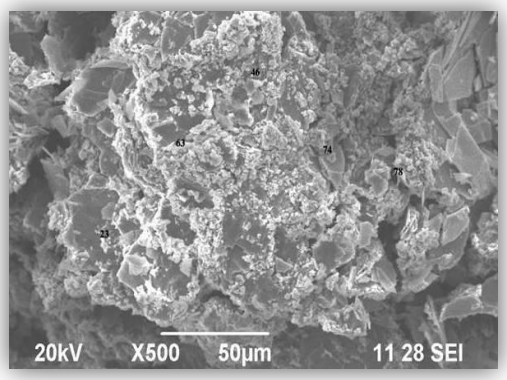


Figure 1: SEM image of Silver nano particle

Figure 2: Antibacterial activity of biologically synthesized silver nano particle

Figure 3: Antifungal activity of biologically synthesized silver nanoparticle

Figure 4: Development of cotton gauze using *Streptomyces griseu* nanoparticle

Table 1: Antibacterial activity of biologically synthesized silver nano particle

Organisms	Streptomyces sp (Culture filtrate)	Streptomyces sp (Culture)	1mM $AgNO_3$	Methicillin
Klebsiellapneumoniae	5mm	1mm	2mm	3mm
Pseudomonas aeruginosa	4mm	1mm	2mm	4mm
Bacillus subtilis	5mm	Nil	1mm	Nil
Escherchia coli	3mm	Nil	Nil	0.5mm

Table 2: Antifungal activity of biologically synthesized silver nano particle

Organism	Streptomyces sp (Culture filtrate)	Streptomyces sp (Culture)	1mM $AgNO_3$
Aspergillusniger	3mm	1mm	Nil

Table 3: Medical Application of biologically synthesized silver nano particle using Gauze cloth

Organism	Streptomyces sp (synthesised filtrate)	Streptomyces sp (Culture)	1mM $AgNO_3$
Pseudomonas aeruginosa	3mm	Nil	Nil

Table 4: Anticancer activity of biologically synthesized silver nano particle

Nm	Control	Test dilution 1 (500 µl)	Test dilution 2 (250 µl)
450 nm	0.274	44.16%	22.12%
540 nm	0.326	64.11%	54.29%

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