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Research Article

BIOSYNTHESIS OF SILVER NANOPARTICLES FROM *STREPTOMYCES SPP* AND ITS ANTIMICROBIAL, ANTICANCEROUS ACTIVITY

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ABSTRACT

Nanotechnology is a field that is burgeoning day by day making an impact in all of human life. Nanotechnology is emerging as a rapidly growing field with its application in science and technology for the purpose of manufacturing new materials at the nanoscale level. The present study deals with the synthesis of silver nanoparticles using actinomycetes isolated from soil. Single colonies were further subjected for the molecular identification. The DNA was isolated and amplified with 16S RNA primers. The gene sequencing was performed for the DNA and identification streptomyces sp. The silver nanoparticle was synthesis using 1mM silver nitrate. The nanoparticles were characterized using UV-Visible study and FTIR the plasmon peak was observed at 430nm confirmed the silver nanoparticle synthesis. SEM was done and the nanoparticle size was measured and found that the size in the same at 60nm to 110nm under 7500X magnification. The antibacterial activity was done against bacteria. The zone of inhibition was maximum against *E.coli and K. Pneumoniae.* The synthesized of nanoparticles were further conjugated with drug Ampicillin (500mg/ml). The anticancer activity was also carried out on He La cell, MTT was done and the cell death was calculated.

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INTRODUCTION

Nanotechnology is an important field of modern research dealing with design, synthesis, and manipulation of particles structure ranging from approximately 1-100 nm in one dimension. Nanotechnology is rapidly gaining importance in a number of areas such as health care, cosmetics, food and feed, environmental health, mechanics, optics, biomedical sciences, chemical industries, electronics, space industries, drug-gene delivery, energy science, optoelectronics, catalysis, reprography, single electron transistors, light-emitters, nonlinear optical devices, and photoelectrochemical applications. (Shuvankar *et al.,* 2012). Recently, biosynthesis of nanoparticles has received attention due to an increasing need of developing a rapid, simple and eco-friendly protocol (A.K.Vala *et al*., 2012). Nanomaterials are seen as a solution to many technological and environmental challenges in the field of solar energy conversion, catalysis, medicine, and water treatment. (Tamasa Panigrahi *et al.,* 2013). Biological synthesis of nanoparticles is a green chemistry approach. Green synthesis of nanoparticles is capable of meeting the requirements of the diverse industrial application. It has been known that silver [Ag] and its compound processes a broad spectrum of antibacterial activities since ancient times. Eco-friendly materials like plant leaf extract, bacteria, fungi, actinomycetes, and yeasts have been used for the green synthesis of silver nanoparticles as it offers numerous benefits (Anima nanda *et al., 2014).* Actinobacteria is gram-positive, aerobic bacteria with high G+C content. They are one of the major groups of soil population and are widely distributed. Actino-bacteria will provide a valuable resource for novel products of industrial interest, including antimicrobial agents (Priya Ragini *et al.,* 2013) silver nanoparticles have been intensely studied because of the distinct properties of their optical behavior, conductivity, chemical stability, and catalytic activity. In addition, silver nanoparticles exhibit broad spectrum bactericidal and fungicidal activity. In addition, silver nanoparticles and antibiotic conjugates can be made efficiently and could result in an increase in the efficacy of antibiotics against various pathogenic, antibiotic-resistant microorganisms. Because of the applications of silver nanoparticles in the fields of biology, environment, and technology, there is a growing need for the development of a cost-effective method for the biosynthesis of silver nanoparticles. (Priyanka singh *et al.,* 2012). The conventional chemical and physical method of synthesis of nanoparticles raise concerns for the negative impact on the environment. This has created a lot of interest in 'Green nanotechnology'. (Devina *et al., 2010).*

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MATERIAL AND METHODS

Collection of Sample

The soil sample was collected for the isolation of actinomycetes from Eachanaari, Coimbatore district. The collected samples were carefully stored in polythene bags and transported to the laboratory for further uses.

Isolation of Streptomyces sp

The isolation was carried out by serial dilution technique on actinomycetes isolation agar medium (HIMDIA). The isolated actinomycetes were identified based on their morphological characteristics.

Sliver Nano Particles Synthesis

The organism was inoculated into sterile LB and incubated at 37°c on a closed rotary shaker for 72 hrs. After incubation, broth was centrifuged at 5000 rpm for 10 min. The pellet and supernatant were collected. The pellet was discaid and the supernatant was collected separately in widal tube. 2 ml of 1mm silver nitrate ($AgNO^3$) was added into 5ml of culture supernatant. Two test tubes were incubated at room temperature in under dark condition for 24hrs. After incubation, the change in color from white to brown was observed.

Characterization of silver Nanoparticles

UV-Vis spectroscopy

The bioreduction of silver ions was monitored by visual observation of colour change to dark brown band further was confirmed by sharp peaks shown by the absorption spectrum of this solution and recorded by using UV-Vis spectrophotometer.

FTIR analysis of silver nanoparticles

The dried powder of AgNPs was subjected to Fourier Transform Infrared Spectroscopy (FTIR) analysis. Two milligrams of the sample was mixed with 200mg KBr (FTIR grad) and pressed into a pellet. The sample pellet was placed into the sample holder and FTIR spectra were recorded in the range 450-4000-500 cm1.

SEM analysis

The morphology of the AgNPs was examined using Scanning electron microscopy (SEM).

Antimicrobial Activity

The antimicrobial activity of AgNPs was tested against *B.subtilis, S.aureus, E. coli, K. Pneumoniae* by the well diffusion method. The wells (10mm) were punched over the agar plates using sterile gel puncher.1mm, 2mm AgNPs, Control $AgNO^3$ and antibiotic disc were added to the wells. The plates were incubated for 24 hours at 37^0C. After incubation, the diameter of inhibitory zones formed around each well was measured in mm and recorded.

Silver NanoConjugate Synthesis

The synthesized silver nanoparticles (10 ml) was coated with Ampicillin (10 ml) and incubated at room temperature for 24 hours. After 24,ms OD was measured at 600 nm to study the efficiency of the drug control.

Isolation of DNA

DNA was isolated by the phenol - chloroform method. Total genomic DNA was extracted from 5 ml overnight NB culture of the purified isolates. PCR was performed in a light cycler Eppendorf PCR machine. A1300 bp fragment was obtained by PCR amplification of the16S rDNA gene using the primers.

Anticancer Activity

He La Cells were grown in RPMI-1640 medium (Hi Media , Mumbai) supplemented with 10% fetal bovine serum (FBS) (Hi Media, Mumbai), 100 U/ml penicillin and 100 µg/m*l* streptomycin (Hi Media, Mumbai) Cells were incubated in a humidified incubator contain 5% CO_2 at 37 ºC.

After 24hrs the cells were seeded into 96 well The cell culture suspension was washed with 1 X PBS (Phosphate Buffered Saline) and then added with 200 µl MTT [3-(4, 5-Dimethyl thiazole-2yl)-2, 5- diphenyl tetrazolium Bromide] solution to the culture flask . It is then incubated at 37°C for 3 hours, removed all MTT solution, washed with 1 X PBS and added with 300 µl DMSO to each culture flask and incubated at room temperature for 30 minutes until all cells get lysed and the homogenous color was obtained. The solution was then transferred to a centrifuge tube and centrifuged at top speed for 2 minutes to precipitate cell debris. Debris was dissolved using DMSO. OD was measured at 540 nm using DMSO blank. Then the percentage viability was calculated using the percentage of viability formulated.

$$\% \text{ of viability} = \frac{\text{OD of Sample}}{\text{OD of Control}} \text{X100}$$

RESULTS AND DISCUSSION

From the analysis, the sliver nanoparticles were synthesized from *Streptomyces* spp. Were isolated and identified from soil sample by serial dilution method. Morphological characterization white milky color colony was produced from actinomycetes isolated agar medium. The synthesis of silver nanoparticles was detected by observing the change in colour of reaction mixture from yellow to brown. The formation of brown colour can be taken as an indication of synthesis of silver nanoparticles as it is known that silver nanoparticles appear brown colour in aqueous solutions due to surface Plasmon Resonance Effect. In the present study culture, supernatant incubated with $AgNO_3$ showed colour change while culture supernatant incubated without addition of $AgNO_3$ showed no change in colour. Thus NPs has been synthesized in the sample and further taken for optical analysis. Vijaya Raghavan *et al.,* (2012) reported the bacterial strain of bacillus sp was inoculated into mineral salts medium the primary conformation of synthesis of nanoparticles in the medium was characterized by the changes in color from white to brown

Synthesis of Silver nanoparticles was confirmed by taking UV-Visible spectra of the reaction mixture after regular time intervals at a range of 300nm-600nm. The spectrum showed a peak at 400 nm which is assigned to SPR effect of silver 430 nm. (Fig 1)

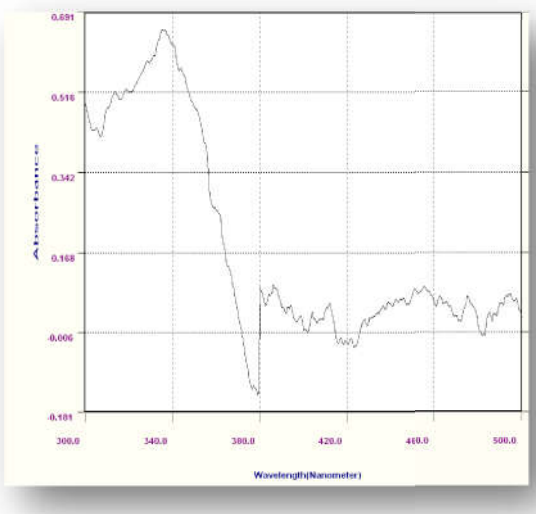

Figure 1 UV analysis-UV specturm

Devina Merin *et al.* (2010) reported that upon addition of Ag+ ions into the cell-free culture in the dark, samples changed the colour from almost colourless to dark brown with intensity increasing during the period of incubation.

Further characterization of silver Nanoparticle was done by FTIR, to identify the possible interaction of biomolecules with silver nanoparticles. FTIR provides the information about functional groups present in the sample which is responsible for the (Fig2) transformation of AgNO3 from simple inorganic to 3 elemental silver. The FTIR studies showed sharp absorption peaks located at 717.52 cm-1 and 3988.79cm-1. The scanning electron micrograph of the silver nanoparticles synthesized after treatment of 1mM silver nitrate solution with aqueous filtrate for 72 hrs, which clearly shows surface deposited silver nanoparticles. The silver nanoparticles synthesized by isolates are irregular in shape.

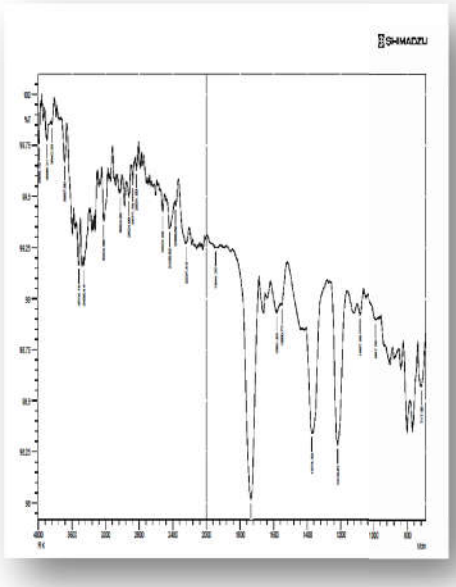

Figure 2 Detection of various groups by FTIR from synthesis of silver nanoparticles of streptomyces sp

Balaji *et al.* (2009) reported FTIR spectroscopic studies on silver nanoparticles obtained from the fungus, *Cladosporium cladosporioides*. Their study confirmed that the carbonyl groups from the amino acid residues and peptides of protein have strong ability to bind silver.

SEM was done and the nanoparticle size was measured and found that the size in the same at 60nm to 110nm under 7500X magnification. Antibacterial effect of nanoparticles and antibiotics was evaluated by well diffusion assay against known human pathogens. The zone of inhibition showed by antibiotics with NPs was relatively larger than the alone antibiotics and NPs. This shows a possibility of synergistic action of nanoparticles and antibiotics. Devika *et al.* (2012) reported that scanning electron microscope surface morphology image showed relatively spherical shape nanoparticles formed with the diameter range 40-50 nm. Nithya *et al.*, (2011) suggested the sliver nitrate has long been considered as a powerful and natural antibiotic and antibacterial agent sliver nanoparticles exhibited antibacterial properties against bacterial pathogens with close attachment of the nanoparticles themselves with the microbial cells

Here the efficacy study of the nanoparticles from streptomyces sp KSIG2 was tested against a microorganism. *E.coli* (6 mm), *B.subtilits* (6 mm), *S.aureus* (3mm), *K. Pneumoniae* (10mm). *E.coli, K.pnenominea* showing high antibacterial activity against the sample. (Table1). Silver nanoconjugates are prepared by combining silver nanoparticles with different biomolecules such as antibody, protein, DNA, drugs, etc. Silver nanoparticles conjugated with antibodies, antibiotics, and various drugs have been extensively used for cancer diagnosis and treatment. The isolated actinomycetes DNA was isolated and the molecular weight was found to be 846kbs. The 16s RNA was done using universal primers and the amplified product was found to be 586kbs. Dreaden *et al*., reported gold nanoparticles, localized in cell line would include DNA damage and cytokinesis arrested. Breast cancer is the most common and the second the leading cause of cancer death among women.

Table 1 Antimicrobial activity of silver Nanoparticles of Streptomyces

BACTERIA	$AgNO_3$	1mm	2mm	RESULT
E.coli	4 mm	9 mm	6 mm	Resistance
S.aurues	3 mm	5 mm	3 mm	Resistance
K. Pneumoniae	3 mm	10 mm	10 mm	Intermediate
B.subtilis	2 mm	3 mm	6 mm	Intermediate

Nanoparticles as silver nanoparticles are usually smaller than several hundred nanometers in size, comparable to large biological molecules such as enzymes, recepotires of a size about 100 to 10000 times smaller than human cells. The cell viability1mM silver nanoparticle 25,50, 75 µl cell death was calculated at 14.29%, 53.72%, 45.72% (Table 2).

Table 2 Anticancer Activity of AgNo3

Sample(1mM silver nano particle)	% of viability	% of cell death
25 µl	85.71%	14.29%
50 µl	46.28%	53.72%
75 µl	54.28%	45.72%

CONCLUSION

Biosynthesized Ag NPs from actinobacteria can be a prominent source for the development of various nanomedicines. This technology had gained so much attention nowadays due to growing demand for environmentally friendly technology for material synthesis.

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