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Synthesis of Silver Nanoparticles from Microbial Source-A Green Synthesis Approach, and Evaluation of its Antimicrobial Activity against *Escherichia coli*

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ABSTRACT

Nanoparticles synthesis by biological methods using various microorganisms, plants, and plant extracts and enzymes have attracted a great attention as these are cost effective, non-toxic, eco-friendly and an alternative to physical and chemical methods. In this research, Silver nanoparticles (Ag-NPs) were synthesized from $AgNO_3$ solution by green synthesis process with the assistance of microbial source only. The detailed characterization of the Ag NPs were carried out using UV-visible spectroscopy, Scanning electron microscopy (SEM), Energy dispersive X-ray Spectroscopy (EDS), Dynamic light scattering (DLS) analysis, and their antimicrobial evaluation was done against *Escherichia coli*. The UV-visible spectroscopy analysis showed the surface plasmon resonance property of nanoparticles. The DLS analysis showed the particle distribution of synthesized silver nanoparticles in solution, and SEM analysis showed the morphology of nanoparticles. The elemental composition of synthesized sample was confirmed by EDS analysis. Antibacterial assay of synthesized Ag NP was carried out in solid (Nutrient Agar) growth medium against *E.coli*. The presence of zone of inhibition clearly indicated the antibacterial activity of silver nanoparticles.

Key words- Antibacterial assay, eco-friendly, nanoparticles, silver nanoparticles, zone of inhibition

1. INTRODUCTION

The National Nanotechnology Initiative (NNI), U.S. defined Nanoparticles as microscopic particles with at least one of the three dimensions less than 100 nm [1]. In recent years, nanoparticles have received enormous attention for the creation and manipulation of products at nanoscale level having novel properties [2]. The properties of nanoparticles entirely differ from conventional macroscopic materials. These unique characteristic properties of nanoparticles arise from the high surface to volume ratio [3].

Silver nanoparticles (Ag-NP) are widely used in huge applications. For many years silver had been used as a colloidal material. But due to development of new techniques for synthesis and characterization, this material has been used in preparation of so many consumer products [4]. Now a day, it is used in many fields like: drug delivery, biosensing, nanodevice fabrication, nanomedicine, imaging, catalysis etc. These applications are due to various properties exhibited by silver nanoparticles like: spectrally selective coating, surface enhanced Raman scattering and antibacterial activity [1]. In addition to above applications, silver nanoparticles (Ag-NPs) can also be used in clothing, sunscreen, cosmetics, and food industry due to its antimicrobial properties. Additionally, the impact of Ag-NPs on environment has been calculated. It is used for waste water treatment. It has been found that when Silver nanoparticles are exposed into waste water, the number of nitrifying bacteria present in sludge is reduced [5].

There are various methods for synthesis of nanoparticles, among them the most popular method is wet-chemical approach [6]. However, these chemical methods are not easily acceptable due to contamination from precursor chemicals, use of toxic solvents, and generation of hazardous by-products. Thus, development of biological synthesis procedure is more favourable, which provides a wide range of environmentally acceptable methodology, low cost of production and minimum time required as compared to wet-chemical processes [7][8]. Recently bacteria and plants are employed for the synthesis of nanoparticles by biological approaches [9].

The main objective of the present research is to present an eco-friendly, one step, cost effective method for production of silver nanoparticles, using the bacterial strain *Bacillus amyloliquefaciens* and evaluation of antimicrobial activity of silver nanoparticles against *Escherichia coli.*

2. MATERIALS AND METHODS

2.1. Materials

$AgNO_3$, required for Silver nanoparticles synthesis, was obtained from Sigma-Aldrich. Other chemicals required for our research purpose were purchased from Merck.

2.2. Source of microorganism

The bacterial strain *B. amyloliquefaciens* was obtained from the Microbiology laboratory of College of Engineering and Technology, Bhubaneswar, Odisha. The obtained pure culture form strain was cultured in nutrient agar (Himedia, Mumbai, India) slant at 37 ^{o}C. It was subcultured from time to time to maintain its viability during the study period.

2.3. Production of biomass

The bacterial strain was cultured in nutrient broth media for biomass production. The culture was incubated on an orbital shaker at 37 ^{o}C and agitated at 100 rpm. After 24 hours of growth, the biomass was harvested and centrifuged at 10,000 rpm for 10 minutes. The supernatant was collected for synthesis of silver nanoparticles.

2.4. Synthesis of silver nanoparticles

The supernatant sample obtained was added separately to the reaction flask containing silver nitrate ($AgNO_3$) at concentration of 10^{-3} M (1%, v/v). The reaction between the supernatant obtained and Ag^{+} ion was carried out in bright conditions for 24 hours. After 24 hours the initial yellowish white colour changed to brown colour, which showed synthesis of Ag nanoparticles [10].

2.5. Antimicrobial activity study

Different materials used for antimicrobial activity assay of silver nanoparticles were nutrient broth, nutrient agar, petri plates, cotton swabs, silver nanoparticles (Ag-NPs), and *Escherichia coli*. To test the antimicrobial activity assay of silver nanoparticles, disc diffusion method was performed.

2.5.1 Preparation of Inoculum

Nutrient broth solution was prepared in a conical flask and it was sterilized. The strain of *E.coli* was added to the conical flask containing nutrient broth. The inoculated bacterial culture was kept on rotary shaker overnight at 100 rpm at room temperature.

2.5.2 Antimicrobial activity assay by disc diffusion method

In order to test the antimicrobial activity of silver nanoparticles, 40 ml of nutrient agar media was prepared and it was autoclaved. Thereafter, it was poured into two petriplates i.e. one to study antimicrobial activity of silver nanaoparticles (Ag-NPs) and other as a control (no nanoparticles was added), and both were kept for 30 minutes for solidifications. 50 µl of fresh overnight culture of *E.coli* was spread over the media. Sterile paper disc made of Whatman filter paper, 4 mm diameter (dipped in nanoparticles sample solution) were placed in one petriplate and other petriplate was used as control (no nanoparticles sample was added). Plates were kept for incubation at 37 ^{o}C for 24 hours to observe the zone of inhibition. The antimicrobial activity of silver nanoparticles formed from different concentrations of $AgNO_3$ like 10^{-3} M, 10^{-4} M, 10^{-5} M was also calculated.

3. CHARACTERIZATION OF SILVER NANOPARTICLES

The Plasmon-resonance property of silver nanoparticles (Ag-NPs) was studied by UV-Vis spectrophotometer (Systronics-117, India). The nanoparticle sample was sonicated first for uniform dispersion and the aqueous component was analyzed at room temperature for Plasmon resonance property.

Jeol-JSM-6480 LV SEM (Germany) machine was employed to characterize the size and shape of silver nanoparticles at an accelerating voltage of 20 Kv. The elemental composition of the synthesized sample was studied by energy dispersive X-ray spectroscopy (EDS).

Laser diffraction method with multiple scattering techniques has been employed to determine the particle size distribution of synthesized sample, which is based on Mie scattering theory. To find out particle size, the sample was dispersed in distilled water followed by sonication. Then the experiment was performed in computer controlled particle size analyser (ZETA sizers Nanoseries, Malvern instruments Nano ZS, UK), to find out particle size distribution.

4. RESULTS AND DISCUSSION

4.1. UV-visible absorption studies

UV-Vis spectra recorded during synthesis ("Fig-1") of nanoparticles shows an absorption maximum at 440 nm, which is typically attributed to plasmon resonance of silver nanoparticles. Due to the tiny dimensions, silver nanoparticles have distinctive color in colloidal solution. The electron cloud at nanometer dimension can oscillate on the surface of the particles and absorb electromagnetic radiation at particular energy. The resonance developed is known as surface plasmon resonance [11]. Depending upon various parameters like: particle shape, size, state of aggregation, and the surrounding medium, the absorption spectrum of nanoparticles show either bathochromic or hypsochromic shift [11].

4.2. Particle size analyser

"Fig-2" shows particle size distribution (PSD) of synthesized silver nanoparticles. The DLS study provides detail knowledge about the particle dispersion, i.e. monodispersed, or polydispersed. However in our study the distribution of particles ranges approximately from 10 nm to 100 nm. From the image, it is confirmed that the sample contains various sizes of nanoparticles, which indeed agrees with the result obtained from SEM analysis.

4.3. Electron microscopy of Ag nanoparticles

Further analysis of Ag-Nps using SEM image showed a clear image of highly dense Ag nanoparticles, which are almost spherical in size ("Fig 3").

4.4. EDS analysis

EDS analysis of silver nanoparticles showed the signal characteristics of elemental silver ("Fig 4"). Due to the surface Plasmon resonance property, silver nanoparticles show absorption band peak approximately at 3 KeV [7][12].

4.5. Antimicrobial activity study

The antibacterial activities of the silver nanoparticles evaluated against *E. coli* are presented in "Fig 5". The result of Silver nanoparticles synthesized from 10^{-3} M concentration of $AgNO_3$ showed moderate antimicrobial activity against *E.coli* with zone of inhibition 7 mm, while the silver nanoparticles synthesized from diluted stock sample (10^{-4} M and 10^{-5} M) showed no zone of inhibition.

There are many factors responsible for the antibacterial activity of Silver nanoparticles. The main mechanism by which these particles showed antibacterial activity might be via oxidative stress generated by reactive oxygen species (ROS). ROS, including superoxide radicals (O^{2-}), hydroxyl radicals (OH^-), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$), can cause damage to proteins and DNA in bacteria [13]. In the present study, metallic silver (Ag) could be the source that created ROS leading to the inhibition of *E. coli*. A similar process was also described by many authors in which zero valent silver (Ag^0) reacted with oxygen to create hydrogen peroxide (H_2O_2) which may damage the bacterial cell wall [13][14][15][16].

5. FIGURES

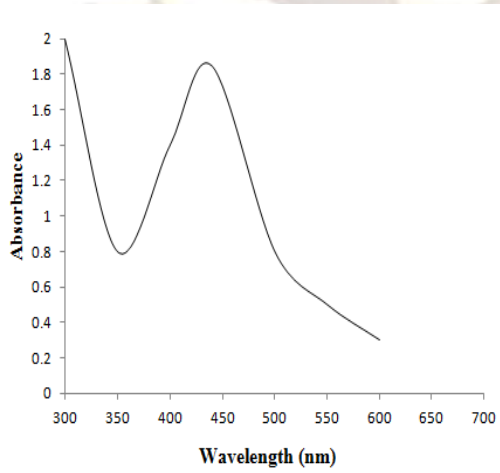


Figure 1. UV-vis absorption spectra of synthesized silver nanoparticles.

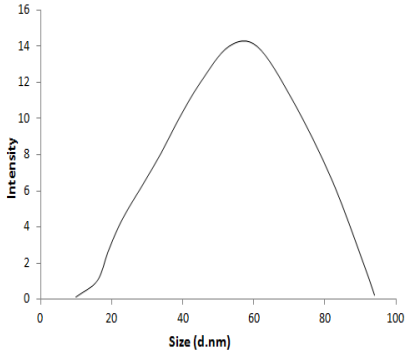


Figure 2: Particle size distribution (PSD) of synthesized silver nanoparticles.

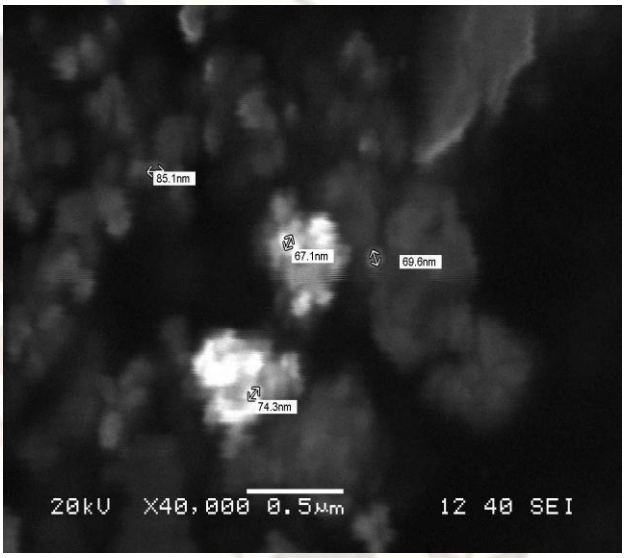


Figure 3. SEM image of synthesized Ag-NPs.

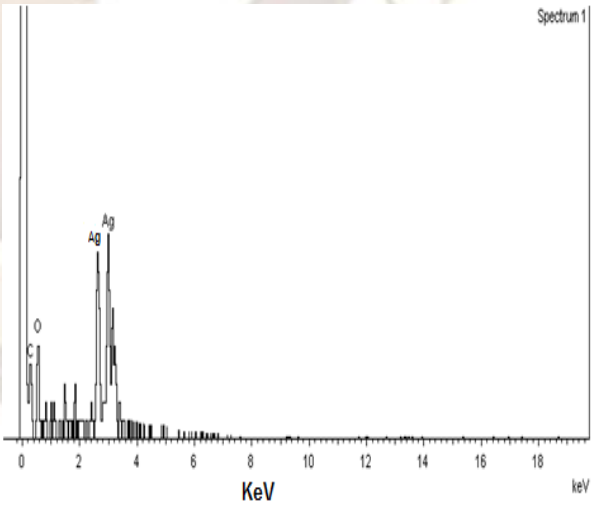
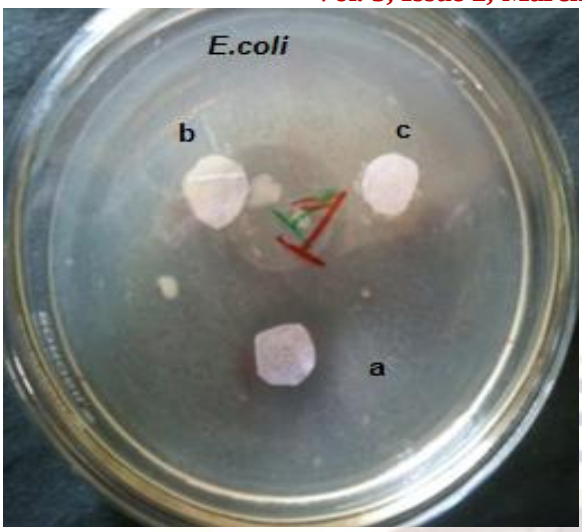


Figure 4. EDS spectra of silver nanoparticles synthesized from microbial source.



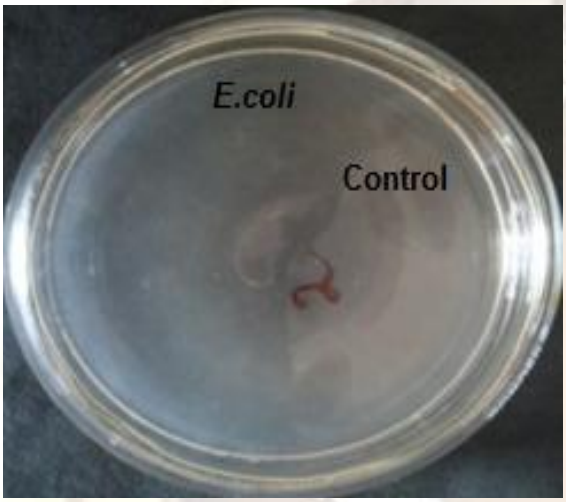


Figure 5. Plate 1 shows antibacterial activity of silver nanoparticles synthesized from different concentrations of $AgNO_3$ (a-10^{-3}M, b-10^{-4} M, c-10^{-5} M). Only at concentration 10^{-3} M of $AgNO_3$, silver nanoparticles shows the zone of inhibition. Plate 2 shows antibacterial activity of control (without nanoparticles.

6. CONCLUSION

The silver nanoparticles were successfully synthesized by using the bacterial strain *B. amyloliquefacien*. The antimicrobial activity of the synthesized silver nanoparticles was confirmed by checking the zone of inhibition in disc diffusion method at 1 mM only, however, lower concentration was unable to induce such activity.

7. ACKNOWLEDGEMENTS

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