Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

European Journal of Experimental Biology, 2015, 5(1):65-70

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Isolation and identification of *E. coli* bacteria for the synthesis of silver nanoparticles: Characterization of the particles and study of antibacterial activity

Akhilesh Kushwaha[1*], Vishal Kumar Singh[1], Juhi Bhartariya[1], Priya Singh[1] and Khadeeja Yasmeen[2]

[1]Institute of Transgene Life Sciences, Lucknow (U.P), India
[2]Genetech Biolabs (P) Ltd., Biotech Park, Lucknow (U.P), India

---

ABSTRACT

The use of microorganisms in the synthesis of nanoparticles emerges as an eco-friendly and exciting approach. In the present study, we report the biosynthesis of silver nanoparticles employing the bacterium E.Coli. The test bacterium was isolated from urine sample grown on EMB Agar medium and identified as E.coli bacteria. Synthesized nanoparticles were characterized by UV-Vis spectroscopy and the maximum absorbance was found to be around 400 nm. The particle size and lattice image of silver nanoparticles was studied by Transmission Electron Microscopy (TEM). The antibacterial activity of these nanoparticles was studied against bacteria Bacillus subtilis and Klebsiella pneumonia. Growth curves of microbes in presence of silver nanoparticles showed inhibition of growth suggesting antibacterial property of the silver nanoparticles.

Keywords; Microbial production of Silver Nanoparticles; *E. coli* bacteria; UV-Vis spectroscopy; Transmission Electron Microscopy; Antibacterial activity; Bacterial growth curve.

INTRODUCTION

Nanotechnology is expected to revolutionize both science and society. Nanotechnology involves tinkering work at atomic levels, tweaking and controlling substances 1, 00, 000 times smaller than a strand of human hair, to make useful materials and devices. It involves technology at the scale of one-billionth of a meter. The term 'NANO' is derived from Greek word "Dwarf" [1]. Nanotechnology is foreseen to significantly influence science, economy and everyday life in 21st century and it may become one of the driving forces to the next industrial revolution. Nanoparticles of Silver have many important applications that include spectrally selective coating for solar energy absorption and intercalation material for electrical batteries, as optical receptors, biolabelling and as antimicrobial agents [2]. The field of nanotechnology is one of the most active areas of research in modern material science. Silver has long been recognized as one of the nanoparticles having inhibitory effect on microbes present in medical and industrial process [3]. Metals nanoparticles such as silver and gold are receiving great interest due to their applications in diverse areas such as medicine, electronics, cosmetics, coatings, packaging, and biotechnology. The synthesis of metal nanoparticles is a more important research branch in nanotechnology. Chemical procedures are

the most widely used methods for synthesis of metallic nanoparticles [4]. In the past few years, the potential of various plants for the synthesis of silver nanoparticles (SNPs) was explored.

Microbial synthesis of nanomaterials utilizes of biological components, primarily prokaryotes and eukaryotes. Microbes play direct or indirect roles in several biological activities. Metals and non-metals present on earth are in constant association with biological components. The most abundant organisms in our biosphere are bacteria. Slight climate changes can potentially be disastrous to the life processes of bacteria; this can result in the prolific advantage for the production of nanoparticles. Many bacterial cultures were used for different kinds of nanoparticles some are gold nanoparticles by *Shewanella* algae it's a kind of marine bacterium and silver nanoparticles by fungus *Verticillium* [5] were also reported. Synthesis of gold and silver nanoparticles by eukaryotic cells such as fungi is reported. Several strains of *Fusarium* viz *Fusarium oxysporum* [6], *Aspergillus fumigatus and Aspergillus flavus* were used for successful production of metal nanoparticles. Recently white rot fungus *Coriolus versicolor* has also been used for the synthesis of stable silver nanoparticles. Biologically synthesized nanoparticles have wide application viz., biosensors biolabelling, in cancer therapeutics and in coating of medical appliances [7].

Among the noble metals, silver (Ag) is the metal of choice in the field of biological system, living organisms and medicine. Green synthesis of nanoparticles is an emerging branch of nanotechnology. In the present study an attempt was made to prepare silver nanoparticles of various concentrations as these particles are fast and specific in their target towards the applications where they are used [8] and evaluate their antimicrobial activity. The most important application of silver and silver nanoparticles is in medical industry such as typical ointments to prevent infection against burnt and open wounds [9]. Silver nanoparticles have diverse applications both in *in vitro* and *in vivo* conditions. A number of approaches such as reduction of solution, chemical and photochemical reactions in reverse micelles [10], thermal decomposition of silver compounds, radiation assisted, electrochemical [11], sonochemical and microwave assisted process [12] and recently via green chemistry route are available for the synthesis of silver nanoparticles.

Silver is a nontoxic, safe inorganic antibacterial agent used for centuries and it has the capability of killing different type of diseases causing microorganisms [13]. Silver has been known to be a potent antibacterial, antifungal and antiviral agent, but in recent years, the use of silver as a biocide in solution, suspension, and especially in nano-particulate form has experienced a dramatic revival. Due to the properties of silver at the nano level, nanosilver is currently used in an increasing number of consumer and medical products. The remarkably strong antimicrobial activity is a major reason for the recent increase in the development of products that contain nanosilver. The main challenge in nanomaterials synthesis is the control of their physical properties such as obtaining uniform particle size distribution, identical shape, morphology, chemical composition and crystal structure.

In this present work, microbial production of silver nano-particles was investigated. Using the bacterial strain *Escherichia coli* as a reducing agent, this research work implies the production of silver nanoparticles and characterization of particles by UV- visible spectrometer, TEM and to study its inhibitory action against bacterial Species *Bacillus subtilis* and *Klebsiella pneumonia.*

MATERIALS AND METHODS

Material

Silver nitrate Merck (Mumbai) India. EMB media, Nutrient agar, LB broth, PDA and PDB used for bacterial growth study was the products of HiMedia, India. Cultures of *Bacillus subtilis and Klebsiella pneumonia* were procured from the Department of Microbiology, Pune.

Sample collection

For the isolation of *E.coli* bacteria urine sample were collected randomly. The collected urine sample was stored in a sterilized glass bottle, maintained at 4°C and then transported to the laboratory with in 1 hour.

Isolation of bacteria

Isolation of bacteria was carried from the "Serial Dilution Method". 10 ml of urine sample was taken in 100ml beaker, and noted as 10^{-1} dilution. From 10^{-1} dilution 1 ml of water was taken using sterile pipette and added to the 9 ml distill water and it noted as 10^{-2} dilution. From the 10^{-2} dilution 1 ml sample was taken and mixed with another 9 ml of sterile distill water and noted as 10^{-3} dilution. This process was continuing up to 10^{-6} dilution. Then 1 ml of

sample was taken from 10^{-3} and 10^{-5} dilution and the sample was spread on Plate (EMB media) to isolate the bacteria *E.coli*. The plate was incubating over night in the incubator at 37°C. It was best method for the isolation of each developing colony in the plate developed from a single cell.

For the isolation of *E.coli* bacteria using EMB Agar media. This media was prepared with composition of Agar 15g, Dipotassium hydrogen phosphate 2g, Eosin Y 0.4g, Lactose 10.0g, Methylene blue 0.065g and Peptone 10g dissolve in 1000 ml distill water. This media is specific for the isolation of *E.coli* bacteria.

Identification

For the identification and characterization of the culture, done morphological (colony color, shape and size) and biochemical tests. The strain was characterized as facultative anarobic, motile, gram negative, nonspore forming bacteria. This strain showed positive result for Catalase test, Indole production, Methyl Red, Macconkey growth, D-glucose acid/gas and D-mannitol fermentation test. They showed negative result with Hydrogen Sulfide (TS1), Oxidase test, Voges Proskauer test, Urea hydrolysis and citrate (simmons) test. The observed characteristics were compared with Bergey's Manual of Determinative Bacteriology for proper identification of the organisms. The species of the strain was identified by Genetech Bio Labs Pvt. Ltd, Lucknow.

Production of biomass

The bacterial strain *Escherichia coli* were cultured in LB medium to produce the biomass for biosynthesis. The culture flask was incubated on an orbital shaker at 37°C and agitated at 200 rpm. The biomass was harvested after 24 hours of growth and centrifuged at 10000 rpm for 10 minutes. The supernatant was collected for further reaction.

Synthesis of silver nanoparticles

For the biosynthesis of silver nanoparticles, 10 ml of supernatant was mixed with 5ml silver nitrate (AgNO3) solution (10 mM) and another reaction mixture without silver nitrate was used as control. The prepared solutions were incubated at 30°C for 24 h. All solutions were kept in dark to avoid any photochemical reactions during the experiment. After 24 hrs as the solution turned into brown from yellow solution. The silver nanoparticles (AgNPs) were purified by centrifugation at 10,000 rpm for 5 min twice, and collected for further characterization.

Characterization of nanoparticles

The appearance of brown color evident that the formation silver nanoparticles in the reaction mixture and the efficient reduction of the Ag ions occurs. The knowledge about the reduction of silver ions and formation of silver nanoparticles were still not clear, but believe that protein molecules and enzyme, includes nitrate reductase enzyme act as good regulating agent in silver nanoparticles synthesis. The formed color solution allowed measuring the absorbance against distinct wave length to conform the formation of silver nanoparticles. The particle size and lattice image of silver nanoparticles was studied by Transmission Electron Microscopy (TEM).

UV-Vis Spectroscopy

Formation of silver nanoparticles is easily detected by spectroscopy because the colored nanoparticle solution shows a peak ~400 nm. In this study, a Shimadzu spectrophotometer was used to measure the optical density of solution.

Transmission Electron Microscopy

Transmission Electron Microscopy was performed by JEOL JSM 1oocx instrument. TEM shows the shape and crystal structure (if any) as well as size of the particles. The grid for TEM analysis was prepared by placing a drop of the nanoparticles suspension on a carbon-coated copper grid and allowing the water to evaporate inside a vacuum dryer. The grid containing silver nanoparticles was scanned by a Transmission Electron Microscope.

Antibacterial activity

Bacterial cultures *Bacillus subtilis* and *Klebsiella pneumonia* were routinely maintained on a Petri dish containing Nutrient agar medium for single colony isolation. To study the antibacterial activity of silver nanoparticle suspension, a fresh colony of each strain was picked up from the Petri plate and suspended evenly in separate tubes containing 10 ml Luria broth. The tubes were incubated for an hour then 1 ml of each culture was inoculated in separate flasks (control and experimental)containing 10 ml LB broth Incubated at 37°C with continuous shaking 150 rpm for 20 hrs. To the experimental flasks, 1 ml of nanoparticle suspension was added; in the corresponding control, nanoparticle suspension was not added.

Growth of bacteria was monitored by measuring the optical density of inoculated growth media at regular time interval (4 hrs) by an UV-Vis Spectroscope at 600 nm. Aliquot from each culture was withdrawn and the optical density was measured and draws the growth curves of bacterial strains.

RESULTS AND DISCUSSION

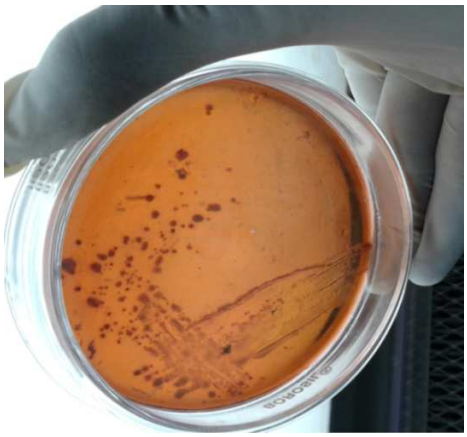

Fig 1: *E.coli* bacterial plate

Synthesis of silver nanoparticles

For the conformation of synthesis of nanoparticles in the medium was characterized by the changes in color of the reaction mixture from light yellow to brown after 24h of incubation. Addition of Ag+ ions to the supernatant, showed the results as color formation to brown,(Fig 2) the color intensity increased with period of incubation due to the reduction in Ag^{o} .Control (without silver nitrate) showed no color formation in the culture when incubated for the same period and condition.

Fig 2: Synthesis of silver nanoparticles

UV-Vis Spectroscopy

The corresponding UV-Vis absorption spectra are shown in Fig 3. The control solution (without silver nitrate solution) shows no evidence of absorption in the range 300 to 900 nm. The samples were exposed to the silver nitrate solution shows the wide spectrum range around 400 nm. The presence of the broad resonance indicates the aggregation of the silver nanoparticles in the solution.

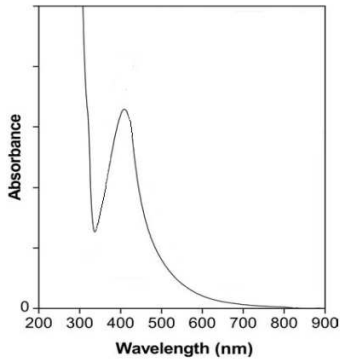

Fig 3: UV-visible absorbance spectra obtained from silver nanoparticles

Transmission Electron Microscopy

TEM analysed the silver nanoparticles coated on carbon coated copper TEM grid. This micrograph showed that they are well-disperse and size ranging from 20-50 nm. The morphology of nanoparticles is essentially spherical.

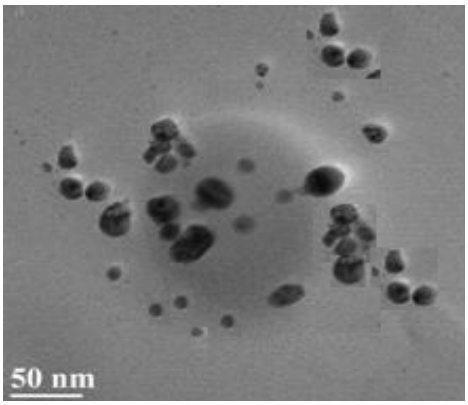

Fig 4: TEM image of Silver Nanoparticles

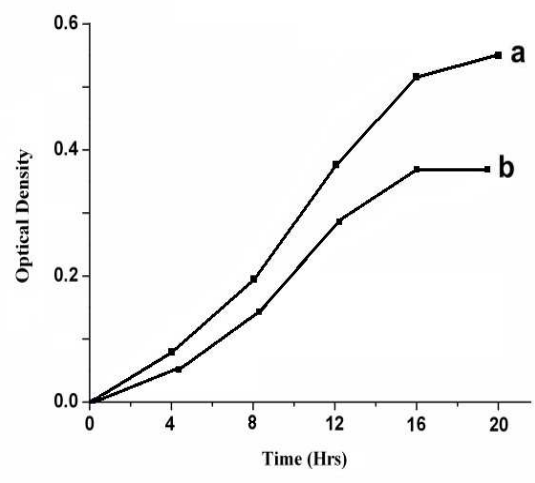

Fig 5: Effect of silver nanoparticles on *B. subtilis*

Antibacterial activity

Figure 5 and 6 shows that in absence of silver nanoparticles the optical density (at λ = 600 nm) of both bacterial cultures (a) increased steadily up to 16 hrs indicating rapid bacterial growth, while in presence of silver

nanoparticles there was a distinct reduction in the growth (b) of both *B. subtilis* and *K. pneumonia* . This confirmed the antibacterial effect of silver nanoparticles on both Gram-positive *B. subtilis* and Gram-negative *K. pneumonia*.

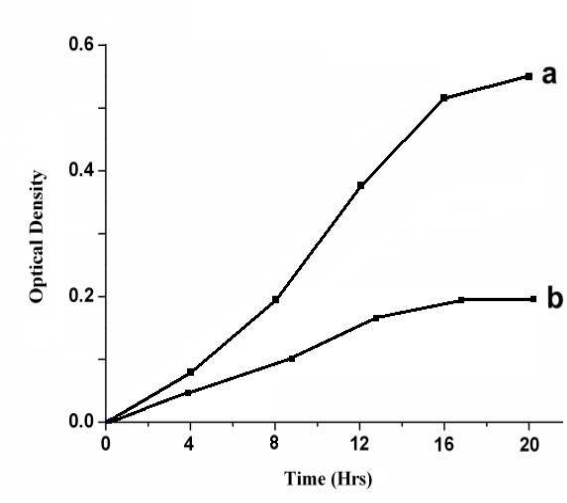

Fig 6: Effect of silver nanoparticles on *K. pneumonia*

CONCLUSION

Microbial synthesis of metal nanoparticles is a reliable and with eco-friendly protocol. The characterization of silver ion exposed to microbial strain and the reduction of silver nitrate to silver nanoparticles was confirmed by UV-Vis Spectrophotometer. The Ag nanoparticles have particle size in the range of 20- 50nm. The mechanism of synthesis of metal nanoparticles by microbes is not clearly explored. However, in this paper it has been taken to understand the possible mechanism of metal and microbes interaction which may be due to structural specificity of the cell of microbes and how metal availability influences microbial resistance.

In this paper we report the coli form bacteria *E.Coli* for synthesis of silver nanoparticles using the culture filtrate. The synthesized nanoparticles have been characterized by UV-VIS and TEM measurements. The nanoparticles proved excellent antimicrobial activity. Hence, the biological approach appears to be cost efficient alternative to conventional physical and chemical methods of silver nanoparticles synthesis and would be suitable for developing a microbial process for commercial large scale-production.

REFERENCES

[1]Parthiban S, Raja S, Mukhopadhyay H K, *Everyman's Science*, **2010**,2,106.
[2]Sharma V, Yungard R, Lin y, *Colloid Interfac*, **2009**, 145, 83.
[4]Jose R M, Jose C L, Alejandra E, Katherine Holt, Juan B K, Jose Tapia R, Miguel Jose Y. *Nanotechnol*, **2005**,16,2346.
[5] Konish Y, Deshmukh N, Tsukiyama T, Saitoh N, *Trans Mater Res Soc Jpn*, **2004**, 29, 2341.
[6]Mukherjee P , Ahmad A , Mandal D , Senapati S, Sainkar R , Khan I , Parischa R, Ajaykumar , Alam M , *Nano Lett*, **2001**, 1, 515.
[7]Duran N, Marcato P, Alves O, Desouza G, Esposito E, *Nanobiotechnol*, **2005**, 3, 8.
[8] Matsumura Y, Yoshikata K, Kunisaki S , Tsuchido T, *Appl Environ Microbio*, **2003**, 69, 4278.
[9] Roy N, Barik A, *Nanotech and Appl*, **2001**, 2, 95.
[10] Ip M, Lui S, Poon K, Lung I, Burd A, *Medical Microbiol*, **2006**, 55, 59.
[11]Taleb C, Petit M, Pileni P, *Chem. Mater*, **1997**, 9, 950.
[12]Rodriguez-Sanchez L, Blanco M, and Lopez Quintela A,. *Phys Chem*, **2000**, 104, 9683.
[13]Pastoriza, S. and Liz Marzan L, *Langmuir* **2000**, 18, 2888.