ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Biogenic Synthesis of Silver Nanoparticles and its Potential Application in Prevention of Acute Ear Infections

Aishwarya Dixit, Satyajeet Das, Anupam Jyoti and Sanket Kaushik

Amity Institute of Biotechnology,
Amity University Rajasthan, Jaipur

Abstract

Objective:
Biogenic synthesis of silver nanoparticles using ear wax microflora and its potential application against ear infection bacteria.

Materials and Methods:
Collection of ear wax sample from healthy volunteer and isolation of prominent colony, which was characterised to identify the microbe. This colony is further used for synthesis of biogenic silver nanoparticles (AgNP). This AgNP is characterised using FTIR, TEM and UV-Vis spec techniques. Anti-bacterial sensitivity test was performed using Kirby-Bauer method against the most prevalent bacteria.

Results:
The UV-VIS absorption spectra of the AgNPs were observed in a range of 300-800 nm. A strong peak specific for the synthesis of silver nanoparticles was obtained at 430nm. Morphological characterization of silver nanoparticles has been previously reported using Transmission electron microscopy (TEM). After the morphological analysis of the nanoparticles was done using TEM it was found that all synthesized AgNPs were spherical in shape with a size approximately 50nm and found to be well dispersed in an aqueous medium. FTIR absorption spectrum of silver nanoparticle powder is 1633.25, 1397.33, 1081.35, 3271.19 cm^{-1}. The antimicrobial activity of the synthesized AgNP was tested against microbiota of the human ear. The zone of inhibition increased with the increase in the concentration of silver nanoparticles and effectively kills them. AgNPs were found to have antibacterial activity against bacteria at 100µg/ml concentration.

Conclusion:
The nanoparticles (AgNPs) were found to be antibacterial against the bacteria. This indicated the potential application of nanoparticles for the prevention of acute ear infections.

Key words: Silver nanoparticles, Biogenic synthesis, ear wax microbiota.

INTRODUCTON:

At the present time, the application of nanostructures in various fields has developed considerably. Nanoparticles have special physicochemical characteristics, such as a high reactivity, high ratio of surface area to mass and sizes in the range of nanometers (10^{-9} m). Nanoparticles have been used in Nano chemistry to increase the immobilization and activity of catalysts, in medical and pharmaceutical nano engineering for distribution of therapeutic agents. They have been used in the food industry also to limit bacterial growth [1–4]. Nanoparticles also possess antimicrobial activity which makes them alternative for antibiotics. Researchers are immensely interested in nanoparticles synthesis by chemical, physical, and biological routes means said as engineered nanoparticles (ENPs) [5]. The biosynthesis of ENPs has great potential with natural reducing agents and stabilizing compounds from bacteria, fungi, yeasts, algae and plants [6, 7]. Besides this, vast applications and rapid utilization of ENPs would assuredly lead to the release of these materials into the environment and different ecosystems. In the past few years, interest in extracellular biogenic synthesis of nanoparticles mainly by bacteria has been increased due to easy synthesis, least toxicity, less downstream processing and better optimization control. A number of bacterial species including *Escherichia coli*, *Staphylococcus aureus*, *Bacillus thuringiensis*, *Corynebacterium* strain SH09 [8-10] etc. have been explored to synthesize AgNPs. The growing resistance of pathogenic bacterial strains to traditional antibacterial treatments has fortified alternate strategies to control infections. In recent years, there has been growing interest in the synthesis and study of silver nanoparticles (Ag-NPs). AgNPs were stated to have antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, methicillin-resistant *Staphylococcus epidermis* (MRSE) etc [11]. AgNPs have the ability to penetrate the bacterial cell wall and then damage the cell membrane that leads to death of the cell. Nanoparticles have protein caps which help in stabilization and binding to cell surface receptor which results in increased binding and uptake of drug or genetic material on human cells [12, 13]. This study deals with the biosynthesis of AgNPs, using cerumen microflora. Furthermore, characterization AgNPs was carried out. Moreover, antimicrobial studies have also been done against the bacterium which is more prevalent in ear infections.

MATERIALS AND METHODS:

Collection and Isolation of Bacteria:

Cerumen micro flora was collected from a healthy volunteer and cultured in Nutrient broth (NB) at 37°C overnight. 100 µl of the broth was then plated on Nutrient agar (NA) plate by spread plate technique and incubated at 37°C overnight. The most numeral and prominent colony was selected and cultured in NB. Further, it was plated in NA, incubated at 37°C overnight and stored at 4°C for further use.

Identification and characterization of bacteria:

Bacteria were identified and characterized by Gram's staining [17], IMViC test [18], catalase test [19] and oxidase test [20].

Synthesis of Silver Nanoparticles (AgNPs) from Bacteria:

The most prominent colony was used for the synthesis of nanoparticles. Single colony of the bacterium was used to inoculate 20ml nutrient broth media. After inoculation the test tubes were incubated at 37°C for 24 hours in incubator shaker. After 24 hours of incubation tubes were centrifuged at 8000 rpm for 10 minutes and the supernatant was transferred in two sterile test tubes, pellet was discarded. 2mM $AgNO_3$ was added to the supernatant of one of the tube under sterilized conditions and the second tube was used as a control. Both the tubes were incubated for 48 hours and the colour change was monitored regularly.

Characterization of Bacterial Silver Nanoparticles:

Analysis by UV-Vis spectroscopy:

As per this method numerous particles ingest ultraviolet or visible light. The rate of transmittance light radiation decides when light of certain recurrence is gone through the tests. Absorption measurements were carried out on SL 164 Double Beam UV-Visible Spectrophotometer. The UV-VIS absorption spectra of the AgNPs were monitored in a range of 300-800 nm for the sample which was used for nanoparticle synthesis. Strong peaks at 420-430 nm were obtained confirming the synthesis of silver nanoparticles.

FTIR spectroscopy analysis:

Fourier Transform Infra-red Spectroscopy (FTIR) is a delicate system helpful for distinguishing natural chemicals in an entire scope of utilizations despite the fact that it can likewise describe some inorganic incorporate paints, glues, pitches, polymers, coatings and medications. It is effective apparatus for detaching and describing natural tainting. FTIR measurements were made to locate the possible biomolecules, which are responsible for the reduction of silver ions to AgNPs and stabilization of AgNPs in colloidal solution. For FTIR analysis synthesized AgNPs were freeze-dried by using Lyophilizer and then mixed with potassium bromide before analysis in the ratio of 1:100. The recurrence extent is measured as wave numbers normally over the reach 4000 – 600 cm^{-1}.

TEM analysis:

To understand the morphology of AgNPs synthesized, the transmission electron microscopic analysis was performed. For TEM measurements, a drop of solution containing synthesized AgNPs was placed on the carbon coated copper grids and kept in infrared light until sample gets dried before loading them onto a specimen holder. TEM micrographs were taken by analysing the prepared grids on Philips CM 200 super twin's TEM instrument operating at 200 kV.

Anti-bacterial sensitivity test:

The antibacterial assays were performed by standard disc diffusion method. Sterile Nutrient agar media was used to cultivate bacteria. The media was poured in the petri discs and kept for 30 minutes for solidification followed by inoculation of overnight cultures of inoculums (50 µl) of *Streptococcus pneumonia* which is a prevalent bacterium in acute ear infections . Sterile paper discs made of Whatman filter paper (6 mm diameter) impregnated with nanoparticle were placed in each plate in triplicate and incubated at 37°C for 20 hrs. After 20 hours of incubation, the zone of inhibition was observed and noted.

RESULTS:

Isolation and characterization of Bacteria

The most numeral and prominent colony was selected and cultured in NB, followed by plating on NA. Prominent pinhead circular whitish colonies were visible throughout the plate. Many dissimilar colonies were used in the study. Bacterial isolate was confirmed by some morphological and biochemical characteristics (Table 1). With reference to the results obtained it may be stated that the colony isolated from the cerumen sample is ***Staphylococcus epidermis*.**

Table 1: Morphological and biochemical characteristics of isolated bacteria

Characters	Isolate
Morphology	
Shape	Cocci
Size	Medium
Gram Stain	Positive
Endospore	Non forming
IMVIC	
Indole	-
Methyl Red	+
Voges Proskauer	+
Citrate	-
Catalase	+
Oxidase	-

Synthesis of AgNPs

After 90 hours the colour of bacterial supernatant containing $AgNO_3$ was changed perfectly from yellow to brown because of reduction of Ag^+ to Ag^0 due to excitation of surface plasmon vibrations in the particles, and thus provides a convenient means of visually determined presence of nanoparticles in sample (Figure 1). There is no change in sample which was used un-inoculated control, it showed the successful synthesis of silver nanoparticles from bacteria.

A B

Figure 1: A**)** Un-inoculated control B) bacterial supernatant containing $AgNO_3$

Characterization of AgNPs

The synthesis of AgNPs was confirmed by UV-VIS spectrophotometer (Shimadzu). The UV-VIS absorption spectra of the AgNPs were observed in a range of 300-800 nm. A strong peak specific for the synthesis of silver nanoparticles was obtained at 430nm (Figure 2). Morphological characterization of silver nanoparticles has been previously reported using Transmission electron microscopy (TEM). After the morphological analysis of the nanoparticles was done using TEM it was found that all synthesized AgNPs were spherical in shape with a size approximately 50nm (Figure 3) and found to be well dispersed in an aqueous medium.

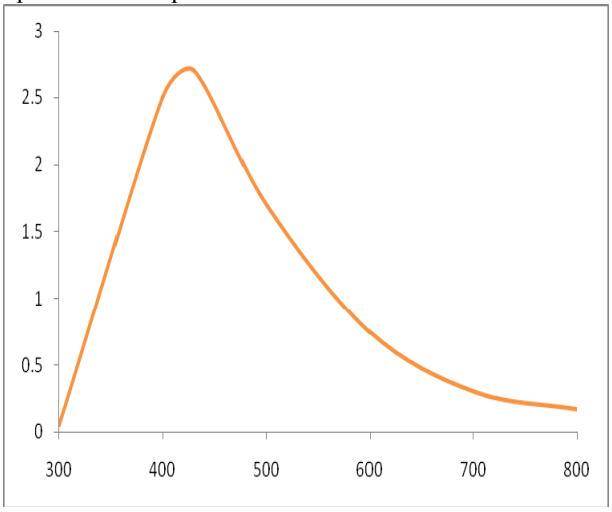


Figure 2: UV–vis absorption spectra of AgNPs

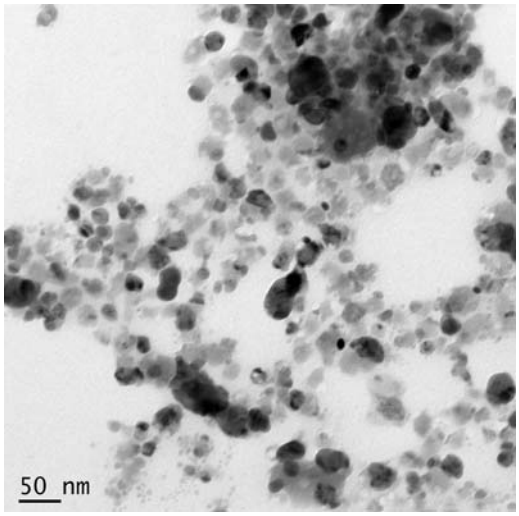


Figure 3: TEM analysis of synthesized AgNPs

FTIR measurement of the dried and powdered sample was carried out to identify the presence of proteins surrounding AgNPs that could be responsible for synthesis and stabilisation of AgNPs. FTIR absorption spectrum of silver nanoparticle powder is shown in figure 4. Absorbance bands analysis in bio reduction and absorbed in the regions is 1633.25, 1397.33, 1081.35, 3271.19 cm^{-1}.

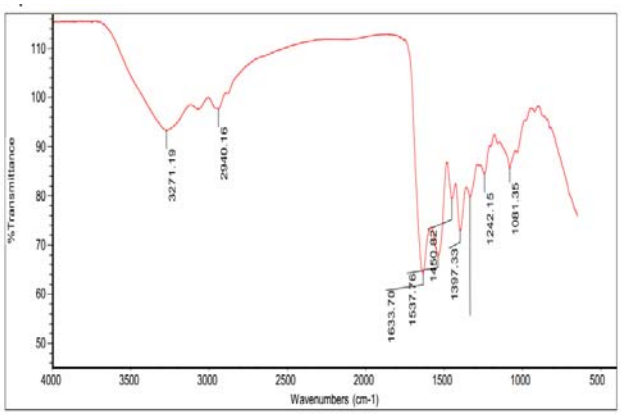
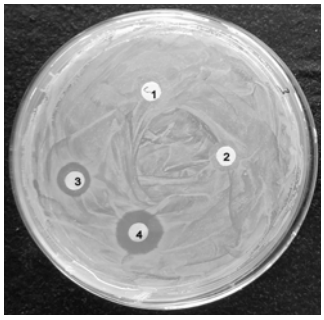


Figure 4: FTIR analysis of AgNPs

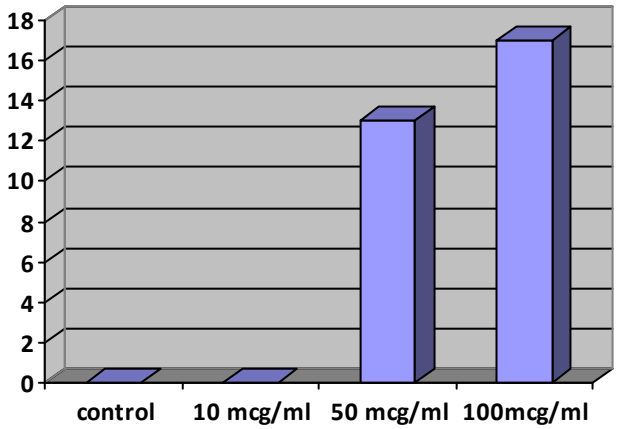
ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES:

Silver and its compounds are known for their antibacterial properties and for treatment of burns, chronic wounds and other bacterial diseases. High surface area to volume ratio cause high bactericidal activity of AgNPs compared with bulk silver metal. The antimicrobial activity of the synthesized silver nanoparticles was tested against microbiota of the human ear. As indicated from the observations the zone of inhibition increased with the increase in the concentration of silver nanoparticles. Therefore these studies are very useful as it shows the effectiveness of green synthesis of silver nanoparticles without any toxic residuals and byproducts. To add to this, antimicrobial activity of the synthesized silver nanoparticles proves the potential application of green synthesis in the area of nano-medicine.



1: Control 2:10µg/ml AgNPs 3:50µg/ml AgNPs 4:100µg/ml AgNPs

Figure 1: Zone of Inhibition



DISCUSSION:

Ear infections are termed as Otitis. Otitis is a very common infection which is found in small children. Generally Otitis is caused by either bacteria or virus. It has already been reported that the leading cause of Otitis are *Streptococcus sp*, non-typeable and *Haemophilus influenzae* [14-16] While considerable work has been done on the treatment of Otitis, a very limited research has been undertaken on the prevention of the infection. In the present study nanoparticles were synthesized from the bacteria isolated by cerumen. The synthesis of nanoparticle was confirmed by the development of brownish colour when the bacterial extract was added. Formation of nanoparticles was further confirmed by UV-VIS spectrophotometer analysis. Silver is known to have antimicrobial properties since a long time. Due to increasing antibiotic resistance, silver has regained interest as an antimicrobial agent. It is previously reported that the antimicrobial activity of AgNPs is more than the antimicrobial activity of silver metal alone. The antibacterial activity of the synthesized nanoparticles was studied against the bacteria which are more prevalent in acute ear infections. The nanoparticles were found to be antibacterial against the bacteria. This indicated the potential application of nanoparticles for the prevention of acute ear infections.

CONFLICT OF INTERESTS:

The authors have no potential conflict of interest regarding publication of the said manuscript.

REFRENCES:

1. Neouze MA. Nanoparticle assemblies: main synthesis pathways and brief overview on some important applications. Journal of Materials Science 2013;48:7321-49.
2. Zhang L, Gu FX, Chan JM, Wang AZ, Langer RS, Farokhzad OC. Nanoparticles in medicine: therapeutic applications and developments. Clinical pharmacology and therapeutics 2008;83:761-9.
3. de Azeredo HM. Antimicrobial nanostructures in food packaging. Trends in Food Science & Technology 2013;30:56-69.
4. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. Biotechnology advances 2009;27:76-83.
5. Kowshik M, Deshmukh N, Vogel W, Urban J, Kulkarni SK, Paknikar KM. Microbial synthesis of semiconductor CdS nanoparticles, their characterization, and their use in the fabrication of an ideal diode. Biotechnology and Bioengineering 2002;78:583-8.
6. Sharma, K.; Kaushik, S.; Jyoti, A. Green Synthesis of Silver Nanoparticles by Using Waste Vegetable Peel and its Antibacterial Activities. Journal of Pharmaceutical Science & Research 2016;8:313-6.
7. Rajora, N.; Kaushik, S.; Jyoti, A.; Kothari, S.L. Rapid synthesis of silver nanoparticles by *Pseudomonas stutzeri* isolated from textile soil under optimised conditions and evaluation of their antimicrobial and cytotoxicity properties. IET Nanobiotechnology 2016;1-7.
8. Nanda A, Saravanan M. Biosynthesis of silver nanoparticles from *Staphylococcus aureus* and its antimicrobial activity against MRSA and MRSE. Nanomedicine: Nanotechnology, Biology and Medicine 2009;5: 452-6.
9. Jain D, Kachhwaha S, Jain R, Srivastava G, Kothari SL. Novel microbial route to synthesize silver nanoparticles using spore crystal mixture of *Bacillus thuringiensis*. Indian J Exp Biol 2010;48:1152-6.
10. Zhang H, Li Q, Lu Y, Sun D, Lin X, Deng X, He N, Zheng S. Biosorption and bioreduction of diamine silver complex by *Corynebacterium*. Journal of Chemical Technology and Biotechnology 2005;80:285-90.
11. Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, Kim SH, Park YK, Park YH, Hwang CY, Kim YK. Antimicrobial effects of silver nanoparticles. Nanomedicine: Nanotechnology, Biology and Medicine 2007;3:95-101.
12. Hu CM, Zhang L, Aryal S, Cheung C, Fang RH, Zhang L. Erythrocyte membrane-camouflaged polymeric nanoparticles as a biomimetic delivery platform. Proceedings of the National Academy of Sciences 2011;108:10980-5.
13. El-Nour KM, Eftaiha AA, Al-Warthan A, Ammar RA. Synthesis and applications of silver nanoparticles. Arabian journal of chemistry 2010;3:135-40.
14. Massa HM, Cripps AW, Lehmann D. Otitis media: viruses, bacteria, biofilms and vaccines. Medical journal of Australia 2009;191:44-9.
15. Arguedas A, Sher L, Lopez E, Saez-Llorens X, Hamed K, Skuba K, Pierce PF. Open label, multicenter study of gatifloxacin treatment of recurrent otitis media and acute otitis media treatment failure. The Pediatric infectious disease journal 2003;22:949-55.
16. Leibovitz E, Jacobs MR, Dagan R. *Haemophilus influenzae*: a significant pathogen in acute otitis media. The Pediatric infectious disease journal 2004;23:1142-52.
17. Gram HC, Friedlaender C. Ueber die isolirte Färbung der Schizomyceten: in Schnitt-und Trockenpräparaten. Theodor Fischer's medicinischer Buchhandlung 1884.
18. Powers EM. U.S. Patent and Trademark Office 1980; U.S. Patent No.4187,351.
19. Walker TK, Tošić J. The catalase test, with special reference to *Acetobacter* species. Biochemical Journal 1943;37:10.
20. Felton LD. Oxidase reaction of various groups of bacteria. The Journal of experimental medicine 1923;38:291-307.