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REVIEW ON BIOSYNTHESIS OF SILVER NANOPARTICLES AND ITS CHARACTERIZATION

Anandalakshmi K .
Department of Physics, Annamalai University, Annamalainagar - 608 002, Tamil Nadu, India
* Corresponding author Mobile: +91-9842694183; E-mail address: anandhi8888@gmail.com

ABSTRACT

The present review focused the biosynthesis of silver nanoparticles (AgNPs) using plant, bacteria and fungi. Green route of AgNPs could be provided the advantage over the physical and chemical synthesis methods. The conventional methods for the production of NPs were expensive, toxic and non-environment friendly. To overcome these problems, many researchers had been found the precise green route. This review described the methods of green synthesis of silver nanoparticles and their mechanism of antibacterial activity.

Introduction

In the recent years, nanoparticles research has played an important role in the modern materials science research. It has attracted considerable interest in the field of electronics, medicine, and biology due to the distinctive properties of nanoparticles such as optical, antimicrobial, cancer therapeutic and catalytic (Jacob *et al.*, 2012, Abdel-Mohsen *et al.*, 2012). Biosynthesis provide advancement over chemical and physical methods as its cost effective, environment friendly, easily scaled up for large-scale synthesis and further there is no need to use high pressure, energy, temperature and toxic chemicals (Jannathul & Lalitha, 2015). Biosynthesized AgNPs are more acceptable for medical applications due to the superior biocompatibility than chemically synthesized ones. Noble metal (gold, silver, platinum and palladium) nanoparticles have been widely used in the electronic, industrial and biomedical fields. Among all the noble metal nanoparticles, silver nanoparticles have attracted intensive research interest owing to their unique properties, such as catalytic and good conductivity, but specifically their wide range of promising bioactivity (Liu *et al.*, 2007, Vardhana & Kathiravan, 2015). Biosynthesis of AgNPs involves bacteria, fungi, yeast and plant extract. Although, many reviews have been published on the fabrication and characterization of AgNPs, limited reports are available on their green synthesis, mechanism of antibacterial activity (Siddiqi & Husen, 2017, Protima *et al.*, 2015, Wei *et al.*, 2015, Siddiqi & Husen, 2016 and Ahamed *et al.*, 2010). Present review describes a comprehensive detail of the biosynthesis of AgNPs from plant extract, bacteria and fungi. In addition, the potential of antibacterial agent and the mechanism of their action have also been discussed. The method of preparation and characterization techniques of AgNPs is summarized in the following Fig. 1.

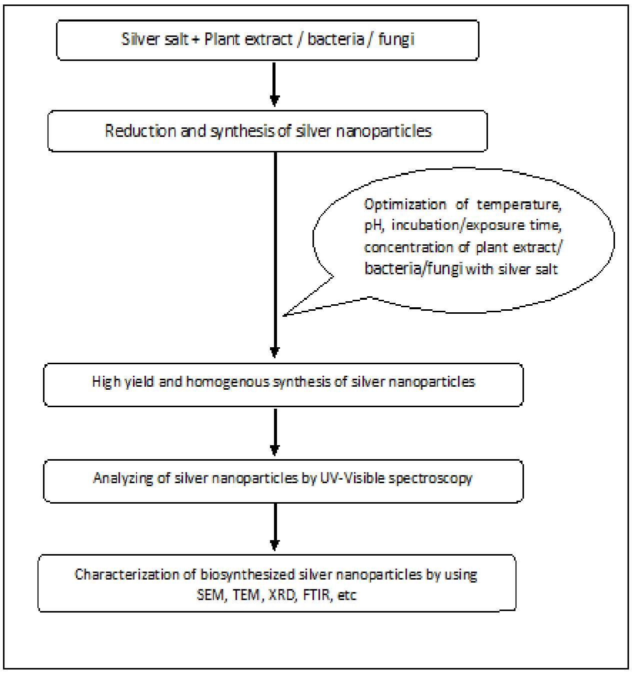

Fig. 1: Method of preparation and characterization techniques of AgNPs

Biosynthesis of AgNPs from plant

Plant related parts such as leaves, flowers, barks, seeds, stems and roots were used for the efficient biosynthesis of silver nanoparticles (Husen & Siddiqi, 2014). Recently, Hemlatha *et al.*, 2020 have reported the biosynthesis of AgNPs using an aqueous leaf extract of *C. Prophetarum*, which act as a capping and reducing agent. The antioxidant activity of the synthesized AgNPs was determined using 2,2-dephenyl-1-picrylhydrazyl (DPPH) and 3-ethylbenzothiazoline-6-sulfonic acid (ABTS). Their antibacterial activity was verified against *staphylococcus aureus* (gram-positive) and *Salmonella typhi* (gram-negative) bacteria. In a recent publication, Fatma Ozturk kup *et al.*, 2020 have reported the synthesis of AgNPs from the leaf of *Aesculus hippocastanum* (horse chestnut) used as the reducing factor to synthesize silver nanoparticles Also, its antioxidant, antibacterial and the application of resveratrol combined AgNPs as efficient delivery vehicles have also analyzed.

Another report showed that the orange peel extract has been used to synthesize AgNPs (Knowarh *et al*., 2011). The orange peel contains abundant amounts of pectin, ascorbic acid, carotenoids, sugar, flavonoids and myriad of flavones, which may act as a reducing agent. During the typical synthesis, the orange peel extract was mixed with 1mM $AgNO_3$ solution under basic condition adjusted by aqueous 2M KOH. The AgNPs size ranged from 3 to 12 nm. The author reported that the ascorbic acid was the reducing agent as shown in the following reaction (Knowarh *et al*., 2011).

$$2Ag^+ + C_6H_6O_6 \longleftrightarrow 2Ag^0 + C_6H_6O_6 + 2H^+$$

The phytosynthesis of AgNPs by Dalbergia spinosa leaves in aqueous extract was investigated by Muniyappan & Nagarajan in 2014. The TEM image showed the average size of AgNPs was approximately 18±4 nm and spherical in shape. They identified carbonyl groups in the reducing sugars, flavonoids and proteins present in the leaves extract. The reducing sugar among the saccharides acted as reductants and the other saccharides acted as protecting agents. The author had evaluated that the AgNPs exhibited considerably enhanced antioxidant and anti- inflammatory activity when compared with the standards of the plant extract. The antibacterial activity of the AgNPs against two gram-negative and two gram-positive bacteria showed moderate antibacterial activity when compared with the standard drug (streptomycin) and the plant extract. These synthesized AgNPs were also effective in the catalytic reduction of 4-nitrophenol (4-NP) into 4- aminophenol (4-AP).

The AgNPs have been synthesized from the seed aqueous extract of *pistacia atlantica*. The UV-Visible peak at 446 nm confirmed the formation of the AgNPs. The crystalline nature of the AgNPs (size 27 nm) was confirmed by the XRD analysis. The FTIR study showed that the extract containing OH functional group acted as a capping in the NPs synthesis. The monodispersed spherical nanoparticles of 10-50 nm size had the better antibacterial activity reported by Sadeghi *et al*., 2015. Nazeruddin *et al*. (2014) represented that AgNPs were synthesized using *Adhathoda vasica* leaf extract. The characteristics of synthesized AgNPs were analyzed by UV-Visible spectrum, XRD pattern and TEM images. The TEM results showed that the NPs were formed in different sizes and the average particle size was found as 11.5 nm. He also studied the anti-microbial activity of AgNPs. Sun *et al*., 2014 had studied that the tea leaf extract can be used as the reducing and capping agent for the synthesis of AgNPs. The synthesized AgNPs were nearly spherical, with the sizes ranging from 20 to 90 nm. In addition, thhe investigated its stability and antibacterial activity.

Synthesis of AgNPs from aqueous extracts of *aloe* and its characterization by UV-Visible spectrum, XRD and TEM had been reported by Zhang *et al*., in 2013. The TEM micrograph displayed that the AgNPs were spherical in shape with an average diameter of around 20 nm. The biosynthesized AgNPs showed good antimicrobial activity against the tested bacterial cultures of *E.coli* and *S. aureus* (Yongqiang zhang *et al*., 2013). Prabakar *et al*., 2013 reported that the AgNPs were synthesized by using leaf extract of *Mukia scabrella*. The formation of the NPs was confirmed by a change in colour of the solution, which revealed a sharp peak at 440 nm in the UV-Visible spectrum. The author investigated that the protein acted as a capping agent with spherical shape NPs of size 18- 21 nm. These AgNPs revealed good antibacterial activity towards the tested pathogens of *Acinetobacter sp. Klebsiella pneumonia* and *Pseudomonas aeruginosa*. The circular shape AgNPs was synthesized by Velmurugan *et al*., 2015 from *Prunus yedoensis* leaf extract and the size of the particles ranged from 20 to 70 nm. The biomolecules in the leaf extract also acted as reducing agent at ambient temperature. These AgNPs exhibited moderate antibacterial activity towards two skin pathogens *P. acnes* and *S. epidermidis*. Table 1 shows the plant mediated synthesis of AgNPs.

Saravana kumar *et al*., 2015 studied that the biosynthesis of AgNPs using leaf extract *cassia fora*. The colourless $AgNO_3$ solution and added leaf extract solution were turned to greenish black indicated the formation of AgNPs. The SPR peak was appeared at 425 nm, which is due to the AgNPs were spherical in shape. The morphology and crystalline nature of the AgNPs were investigated by SEM and XRD analysis. The functional groups of C=O, N-H and O-H groups were incorporated in the reduction of Ag^+ to Ag^o. 2,2-Diphenyl-1-picrythydrazyl (DPPH) radical scavenging activity tends to increase with increasing the concentration of Ag NPs. The synthesized AgNPs had revealed significant antibacterial activity against *E.coli*, *P. aeruginosa*, *S. aureus* and *B. Subtilies*. The better inhibition activity was observed in the *E. coli*. The AgNPs are synthesized by utilizing *Pedalium murex* leaf extract as capping as well as reducing agent

Table 1: Plant mediated synthesis of AgNPs

Plant	Plant parts	Nanoparti cles size	Phyto constituent responsible for reduction of AgNPs	References
Sida cordifolis	leaf	10-30 nm	Alkaloids, quinazolines, cryptoleptins, phytosterols, flavonoids and saponins	Srinithya *et al.*
Lxora coccinea	flower	5-10 nm	Alkaloids, tannins,glucosides, flavonoids, saponins, terpenes and carbohydrates	Nalvolthula *et al.*
Pongamia pinnata	seed	5-30 nm	Pongaflavanol, tunicatachalcone, pongamol, galactoside and glybanchalcone	Beg *et al.*

Fraxinus excelsior	leaf	25-40 nm	Flavonoids, alkaloids, glycosides, terpenoids, phenolic compounds, amino acid residues and peptides of proteins.	Parveen *et al.*
Emblica officinalis	fruit	10-70 nm	Alkaloids, phenolic compounds, amino acids and tannins	Ramesh *et al.*
Putranjiva roxburghil	leaf	5.74 nm	Amino groups	Ali *et al.*
Areca catechu	nut	18.2 and 24.3 nm	Polyphenols	Rajan *et al.*
Lycium barbarum	fruit	3-15 nm	Tannias, flavonoids, ascorbic acid and alkaloids	Dong *et al.*
Ficus benghalensis and Azadirachta indica	bark	60 nm	Flavonoids, terpenoids and phenols	Nayak *et al.*
Syzygium alternifolium	fruit	4-48 nm	Phenols and primary amines of proteins	Yugandhar *et al.*
Salvinia molesta	leaf	12.46 nm	Alkaloids, flavonoids, tannins, phenols, sugar and proteins	Verma *et al.*
Helicteres isora	root	16-95 nm	Steroids, terpenoids, alkaloids, carbohydrates and phenolic compounds	Bhakya *et al.*
Afzelia quanzensis	bark	10-80 nm	Protenis	Moyo *et al.*
Alpinia calcarata	root	5-15 nm	Proteins, flavonoids and polyphenols	Pugazhendhi *et al.*
Diospyros paniculata	root	17 nm	Phenolics and proteins	Rao *et al.*
Solanum indicum	leaf	10-50 nm	Phenolic compounds	Sengottaiyan *et al.*
Aristolochia indica	leaf	32-35 nm	---	Shanmugam *et al.*

by adding to the aqueous solution of $AgNO_3$. The properties of the AgNPs are analyzed by UV-Visible spectrometer, X-ray diffractometer (XRD), Fourier Transform infrared (FTIR) spectrophotometer, and Transmission electron microscopy (TEM). The TEM micrographs suggested that the size of the particles were around 50 nm and spherical in shape. The synthesized AgNPs had the highest antibacterial activity against *E. coli* and *B. subtilis*. These biosynthesized nanoparticles are widely used in the cancer therapy, wound healing, antimicrobial activity, water paints, cotton fabrics and textiles etc. The biosynthesized AgNPs has also paved a better methodological approach in the medical field (Anandalakshmi *et al*., 2016).

Biosynthesis of AgNPs from fungi

Dattu singh *et al*., 2013 reported that the use of endophytic fungi as a biological system, environmental friendly, effective, and also have the ability to reduce metals for synthesis of nanoparticles production. They reported that the proteins with nanoparticles play an important role in stabilization and as reducing agent. Antibacterial activity against *ps. Aeruginosa* and *K. pneumonia* showed maximum zone of inhibition of 21 and 15mm respectively. The TEM images showed the synthesized AgNPs was spherical in shape, well dispersed with the size of 25 nm, using the fungi *Sclerotinia sclerotiorum* MTCC 8785 strain, 25-30 nm (Juhi *et al.,* 2016) and 1-20 nm (Li *et al*., 2011) using the fungus *Aspergillus flavus* with the average particle size was 8.92±1.61nm (Vigneshwaran *et al.,* 2007). Fungi can be used through various methods for synthesis of nanoparticles in which, fungi secrete variety enzymes which are used to reduce $AgNO_3$ solution (*Mandal et al., 2006*). Kuber *et al*., 2006 have reported that the extracellular synthesis of AgNPs from fungus *Aspergillus fumigatus*. The synthesis process was quite fast and AgNPs were formed within a minutes of silver ion coming in contact with the cell filtrate. The TEM micrograph showed formation of well dispersed AgNPs in the range of 5-25 nm. It is a simple process for the extracellular synthesis of AgNPs from silver nitrate solution (i.e through the reduction of Ag^+ to Ag^o) using *Fusarium semitectum*. The TEM image indicated that the particle size from 10 to 60 nm and are mostly spherical in shape (Basavaraja *et al*., 2008) using fungus *Fusarium oxysporum*, 5-15 nm (Absar Ahmad *et al*., 2003).

Intracellular synthesis of AgNPs from *Aspergillus terrerus* (MTCC632) was carried out by Sulbha Girish Velhal *et al*., 2016. The formation of AgNPs depended on the factors, temperature, amount of biomass and concentration of silver ions in the reaction mixture. The antibacterial property was demonstrated by fabric embedded with AgNPs. The silver nano-embeded fabric has excellent antibacterial properties and could be applied in large scale. The extracellular synthesis of silver nanoparticles by using *F. oxysporum* and its antibacterial effect on textile fabrics was investigated by Duran *et al*., 2007.

Biosynthesis of AgNPs from bacteria

Vidhyalakshmi Das *et al*., 2014 investigated that the synthesis of AgNPs by a bacterial strains (CS11). On treating the bacteria with 1mM $AgNO_3$, it was found to have the ability to form the silver nanoparticles extra cellular at room temperature within 24h. The colour of the solution changed from pale yellow to brown in colour. The absorption spectrum of AgNPs exhibited a strong broad peak at 450nm. The TEM result revealed that the AgNPs were spherical in shape and the size range 42-94 nm. Saravanan *et al*., 2017 have reported that the synthesized AgNPs using the bacterial *exopolysaccharide* (Eps). The reduction of Ag^+ ions with Eps to form AgNPs was observed after 1, 5, 10, 20 and 30 days of incubation. The colourless solution changed to dark yellowish brown in colour. The bacterial Eps acted as both a reducing and stabilizing agent. The TEM images revealed that the nanoparticles have spherical shape with the size of 35 nm.

Tomer *et al*., 2019 have reported the biosynthesis of AgNPs using an aqueous extract of the *Cyanobacterium Haloleptolyngbya alcalis* KR 2005/106 from a soda lake. The extract acted as a reducing agent for AgNPs synthesis and resulted formation of nanoparticles < 50 nm in size. Shahverdi *et al*., 2007 have also reported the rapid (< 5 min) synthesis of AgNPs using culture supernatants of *K. pneumonia, E. coli* and *Enterobacter cloacae*. The optimized reaction parameters for efficient synthesis of AgNPs were biomass quantity of 80µg/m, pH 5.5, 60 ^{o}C temperature, duration of 60min UV light exposure and 1mM $AgNO_3$ solution. The biosynthesized AgNPs has a spherical shape with the size of 60-80 nm (Husain *et al.,* 2019).

Samadi *et al*., 2009 have demonstrated an intra/extracellular biosynthesis of AgNPs using bacteria *Proteus mirabillis* PTCC1710. The selection of bacteria makes biosynthesis effective, flexible, reasonable and a suitable method for large- scale synthesis. The TEM images showed the formation of the spherical shape of the particles in the size of 10-20 nm using *Psychrophilic* bacteria (*Shivaji et al.,* 2011). The biosynthesis was rapid and AgNPs were formed within few minutes if AgNPs comes to contact with cell filtrate. Extracellular synthesis of AgNPs using Bacillus megaterium (NCIM 2326) strain cultured supernatant, further synthesized AgNPs to determine the antibacterial activity on multi drug resistant clinical pathogens, such as *Streptooceus pneumonia* and *Salmonella typhi* was performed by Saravanan *et al.,* in 2011. Quinteros *et al*., 2016 demonstrated that the AgNPs generated oxidative stress in *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* mediated by the increment of reactive oxygen species and that was increase correlated with better antimicrobial activity.

Priyadarshini *et al*., 2013 have reported that the synthesis the AgNPs from *Bacillus strain S-27*, belonging to *B.flexus* group and 1mM $AgNO_3$ in aqueous medium. The colourless solution turned yellow and finally formed brown in colour. The UV-Visible spectrum exhibited a peak at 420 nm due to surface plasmon resonance (SPR) of AgNPs. Anisotropic nanoparticles, such as spherical and triangular shaped NPs, sizes were found 12 and 65 nm. These AgNPs were demonstrated to be effective towards multidrug resistant gram positive and gram negative bacteria using bacterial *exopolysaccharide*. Multi dispersed AgNPs were formed different shape, mean diameter of 100 nm ranging from 2 to 15 nm. The fungi *Aspergillus spp* has better zone inhibition than *Penicillum spp* (Kanmani & lim, *2013*).

Mechanism of antibacterial activity of AgNPs

Silver nanoparticles have numerous antifungal and antimicrobial applications. As discussed earlier, many reports are available which have shown that AgNPs are effective against pathogenic organisms namely *S.aureus , B.subtilis, Syphilis typhus, P.aeruginosa, E. coli, Vibrio cholerae S. entrica* etc (Singh *et al*., 2020, Ouay *et al*., 2015, , Ajitha *et al.,* 2015, Nakkala *et al*.,2014 & Parthiban *et al*., 2019). The highest bactericidal activity is certainly due to the silver actions released from AgNPs that act as reservoirs for the Ag^+ bacterial agent. Big changes in the membrane structure of bacteria as a result of the interaction with silver cations lead to the increased membrane permeability of the bacteria (Das *et al*., 2013; Dibrov *et al.,* 2002; Sondi & Salopek Sondi*,* 2004). The AgNPs with larger surface area provide a better contact with microorganisms (Rai *et al.,* 2009 & Ibrahim*,* 2015). Lok *et al*., 2006 explained that AgNPs exhibited destabilization of the outer membrane and rupture of the plasma membrane, thereby causing depletion of intercellular ATP (81). On the other hand, Sarkar *et al*., 2007 also elucidated that for *S.aureus*, the AgNPs demonstrated better bactericidal efficiency compared to penicillin. Yu-Sen *et al*., 1997 reported that Ag ions from AgNPs are believed to become attached to the negatively charged bacterial cell wall and rupture it, which leads to denaturation of protein and finally cell death (Lin *et al.,* 1998). Another mechanism that has been explained is that the R-S-S-R bonds are formed through reactions related to silver and oxygen with sulfhydryl group on the cell wall, therefore blocking respiration and causing cell death (Kumar *et al.,* 2004 & He *et al.,* 2017). Thus, the AgNPs are capable to penetrate the cell membrane or attach to the bacterial surface based on their size. Smaller particles having the larger surface area available for interaction will give more bactericidal effect than the larger particles (Baker *et al*., 2005 & Morones *et al.,* 2005).

Amro *et al*., 2000 demonstrated that metal depletion may cause the formation of irregularly shaped pits in the outer membrane and change membrane permeability, which is caused by the progressive release of lipopolysaccharide molecules and membrane proteins. Moreover, antibacterial activity may be related to the small size and high surface area of the developed AgNPs, which permit them to reach easily the nuclear content of bacteria Reicha.*et al.,* 2012 & Chun-Nam Lok, 2006). The pathogenic effect of the NPs can be attributed to their stability in the medium as colloid, which modulate the phosphotyrosine pattern of the pathogen proteins and consequently arrests their growth. However, it was proposed that the variation in growth inhibition of bacteria by NPs may be attributed to the presence of peptidoglycan, which has a strong negative complex structure (Ahmad *et al., 2011*). Cui *et al*. (2016) suggested that the mechanism of antimicrobial activity is due to by free radicals generation, where the AgNPs was interacted with bacteria, consequently release the silver ions inside of the cell and the contents of the cells leaked out, eventually, if leads to the protein denaturation.

Tamboli and Lee, 2013 elucidated that the high tendency of silver particles to sulfur and phosphorus is the main reason of its antibacterial properties. Sulfur and Phosphorus are found abundantly throughout in the membrane of the bacterial cell. The AgNPs react with proteins containing sulfur inside or outside of the cell membrane which effects on the survival of cells. Rautela *et al*., 2019 evidently proved that the Gram negative bacteria (*E.coli*) showed a higher zone of inhibition, in comparison to gram positive bacteria (*B. cereus and S. aureus*) at the same concentration of AgNPs. This variation could be explained by differences in the composition of the cell wall Gram-positive and Gram- negative bacteria. In Gram-positive bacteria, the cell wall is constituted of a thick peptidoglycan layer, consisting of short peptide cross- linked linear polysaccharide chains. This leads to a more rigid structure, increasing difficulties in penetration of the silver nanoparticles. On the other hand, the gram negative bacteria's cell wall is composed of a thin peptidoglycan layer (Rautela *et al.,* 2019 & Shrivastava *et al.,* 2007).

Conclusion

An increasing awareness towards bio synthesis and the use of green route for synthesis of silver nanoparticles leads to a desire to develop environmental friendly technique. The reduction silver ions by bacteria, fungi and plant extracts resulted in the formation of stable nanoparticles with multi shaped morphologies resulted in >100nm size range of silver nanoparticles. Use of green chemistry for synthesis of AgNPs is an emerging and exciting area of nanotechnology and may have a significant impact on further advances in nanosciences. The fabrication of AgNPs is used as an electrochemical sensor, antimicrobial agent, in medicine, biosensors, health care, agriculture and biotechnology. Other advantages of green synthesis from plant derivatives are provision of a hygienic working environment, environment shielding and health, lesser wastages and most stable products.

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