Review

Biosynthesis of silver nanoparticles

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The use of microorganisms in the synthesis of nanoparticles emerges as an eco-friendly and exciting approach. Several microorganisms have been known to produce silver nanoparticles (Ag NPs), when silver molecules are exposed either intracellularly or extracellularly. Intracellular synthesis may accomplish a better control over the size and shape distributions of the nanoparticles, product harvesting, and recovery are more cumbersome and expensive. The extracellular synthesis by comparison is more adaptable to the synthesis of a wider range of nanoparticles systems. These silver nanoparticles are found to play a major role in the field of nanotechnology and nanomedicine. This review is thus an overview of Ag NPs, biosynthesis by biosynthetic methods such as biological microorganisms (bacteria and fungi) and plants extract and their advantages.

Key words: Nanotechnology, silver nanoparticles, biological synthesis, antimicrobial agent.

INTRODUCTION

The word "nano" is used to indicate one billionth of a meter or 10^{-9}. The term nanotechnology was coined by Professor Norio Taniguchi of Tokyo Science University in the year 1974 to describe precision manufacturing of materials at the nanometer level. Research in the field of nanotechnology mostly deals with the synthesis and stabilization of various nanoparticles by physical and chemical processes. Currently, there is a growing need to develop an eco-friendly process for nanoparticle synthesis and hence the focus turned towards 'green' chemistry and bioprocesses. 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 (Albrecht et al., 2006). These nanomaterials are already having an impact on health care. Now-a-days we are using nanoproducts in various fields.

Nanoparticles are clusters of atoms in the size range of 1 to 100 nm. "Nano" is a Greek word synonymous to dwarf meaning extremely small. The use of nanoparticles is gaining impetus in the present century as they posses defined chemical, optical and mechanical properties. The metallic nanoparticles are most promising as they show good antibacterial properties due to their large surface area to volume ratio, which is coming up as the current interest in the researchers due to the growing microbial resistance against metal ions, antibiotics and the development of resistant strains (Gong et al., 2007). Nanomaterials often show unique and considerably changed physical, chemical and biological properties compared to their macro scaled counterparts (Li et al., 2001). Synthesis of noble metal nanoparticles for applications such as catalysis, electronics, optics, environmental, and biotechnology is an area of constant interest (Hussain et al., 2005; Burleson et al., 2004; Cheng, 2005; Albrecht et al., 2006). Gold, silver, and copper have been used mostly for the synthesis of stable dispersions of nanoparticles, which are useful in areas such as photography, catalysis, biological labeling, photonics, optoelectronics and Surface Enhanced Raman Scattering (SERS) detection (Kearns

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et al., 2006). Additionally, metal nanoparticles have a surface plasmon resonance absorption in the ultra-violet (UV)-visible region. The surface plasmon band arises from the coherent existence of free electrons in the conduction band due to the small particle size (Burda et al., 2005; Tessier et al., 2000).

Generally metal nanoparticles can be prepared and stabilized by physical, chemical and biological methods; the chemical approach, such as chemical reduction, electrochemical techniques, and photochemical reduction is most widely used (Frattini et al., 2005). Moreover, the nanoparticles of noble metals, functionalized, biocompatible and inert nanomaterials are found to have potential applications in microelectronics (Li et al., 1999), optical devices (Kamat, 2002), catalysis, drug delivery system (Mann and Ozin, 1996) and cancer diagnosis and therapy (Sengupta et al., 2005; Gao et al., 2004; Singh et al., 2008). The target delivery of anticancer drugs has been done using nanomaterials (Sengupta et al., 2005). With the use of fluorescent and magnetic nanocrystals, the detection and monitoring of tumor biomakers have been demonstrated (Gao et al., 2004). Silver nanoparticles, which are used as disinfectant, have some risks as its exposure to silver can cause agyrosis and argyria also; it is toxic to mammalian cells (Gong et al., 2007). The current investigation supports that use of silver ion or metallic silver as well as, silver nanoparticles can be exploited in medicine for burn treatment, dental materials, coating stainless steel materials, textile fabrics, water treatment, sunscreen lotions, etc. and posses low toxicity to human cells, high thermal stability and low volatility (Duran et al., 2007).

The studies have shown that the size, morphology, stability and properties of the metal nanoparticles are strongly influenced by the experimental conditions, the kinetics of interaction of metal ions with reducing agents, and adsorption processes of stabilizing agent with metal nanoparticles (Sengupta et al., 2005; Knoll and Keilmann, 1999). Hence, the design of a synthesis method in which the size, morphology, stability and properties are controlled has become a major field of interest (Wiley et al., 2007).

SILVER AS ANTIMICROBIAL AGENT

Since ancient times, silver has been used for disinfecting stored water and liquids. The ancient Greeks and early Americans used silver coins for this purpose. Historical review reveals silver being used to treat maladies. Prior to the 1800s, silver was used for treating epilepsy, venereal infections, acne, and leg ulcers. Silver foil applied to surgical wounds was known to improve wound healing and reduce post operative infections, and silver pencils were used to remove warts and to debride ulcers. In the late 19th century, 1% $AgNO_3$ solution was instilled into conjuntiva sacs to reduce post partum eye infections. In

the late 1960s, Moyer and Monafo introduced silver nitrate 0.5% solution for burn wound treatment (Dunn and Edwards-Jones, 2004). However, silver nitrate dressings are labour intensive as they need to be applied several times a day or re-moistened two hourly. The potency of silver as an antimicrobial was found to be related to the amount and rate of free silver released onto the wounded. In the late 1960s, Fox introduced silver sulfadiazine cream for burn wound management. This dramatically revolutionized the management of burn wounds by reducing the incidence of burn wound infections. Silver sulfadiazine cream has a relatively short action, its penetration of the burn eschar is poor and it forms a pseudo-eschar. Both silver nitrate dressings and silver sulfadiazine cream require a high frequency of dressing changes (Joy and Fiona, 2006).

Infection is the most common complication and cause of death in patients. Therefore, antibacterial effects of Ag have been incorporated into various medical applications. Plastic catheters coated with silver nanoparticles (Ag NPs) prevent biofilm formation from *Escherichia coli, Enterococcus, Staphylococcus aureus, Candida albicans, Staphylococci* and *Pseudomonas aeruginosa* and also show significant results in *in-vitro* antimicrobial activity (Roe et al., 2008). Silver aerosol NPs were efficient as antimicrobial agents against *B. subtilis* (Yoon et al., 2008). Supplementation of Ag NPs with antibiotics as penicillin G, amoxicillin, erythromycin, clindamycin, and vancomycine against *E. coli* and *S.aureus* has been examined. The presence of Ag NPs increased the antibacterial activities of antibiotics for both strains (Shahverdi et al., 2007). Additionally, Ag NPs-embedded paints demonstrated killing of both Gram positive human pathogens and Gram negative bacteria (Kumar et al., 2008).

The antimicrobial property of silver is related to the amount of silver and the rate of silver released. Silver in its metallic state is inert but it reacts with the moisture in the skin and the fluid of the wound and gets ionized. The ionized silver is highly reactive, as it binds to tissue proteins and brings structural changes in the bacterial cell wall and nuclear membrane leading to cell distortion and death. Silver also binds to bacterial DNA and RNA by denaturing and inhibits bacterial replication (Lansdown, 2002). Silver sulfadiazine (AgSD) is a combination of silver and sulfadiazine. AgSD is used as a 1% water-soluble cream. AgSD works as a broad spectrum antibiotic. It is used especially for the treatment of burn wounds. AgSD binds to cell components including DNA and cause membrane damage (Atiyeh et al., 2007). It achieves bacterial inhibition by binding to the base pairs in DNA helix and thus inhibits transcription. In similar way it also binds to phage DNA (Fox and Modak, 1974; Mcdonnell and Russell, 1999).

The silver nanoparticles were synthesized using *Klebsiella pneumoniae* and evaluated its antimicrobial activity against *S. aureus* and *E. coli* was evaluated. The antibacterial activity of antibiotics like penicillin G, amoxicillin, erythro-

mycin, clindamycin and vancomycin was pronounced in the presence of silver nanoparticles against *E. coli* and *S. aureus*. The highest synergistic activity was observed with erythromycin against *S. aureus*. Synthesis of silver nanoparticles in the size range of 10 to 15 nm and its dose dependent effect is on the Gram negative and Gram positive bacteria. From the results it was found that the dose dependent silver nanoparticles have marked activity against gram negative organisms than the gram positive organisms (Shrivastava et al., 2007).

The effect of Ag NPs on bacterial growth of E*. coli, Vibrio cholera, P. aeruginosa*, and *Syphillis typhus* has been studied using a high angle annular dark field (HAADF) scanning transmission electron microscopy (STEM) technique (Morones et al., 2005; Sondi et al., 2003). This technique can identify presence of Ag NPs as small as 1 nm. Above 75 µg/ml of Ag NPs concentration, there was no significant bacterial growth observed. Some noticeable damage to the cell membrane by Ag NPs could be seen and the damage to cell may be caused by interaction of Ag NPs with phosphorous and sulfur containing compounds such as DNA. Silver tends to have a high affinity for such compounds (Basu et al., 2008). Furthermore, the antibacterial activity of Ag^+ ion under anaerobic conditions was found less potent than in oxygen rich environment. Such interactions in the cell membrane would prevent DNA replications (Matsumura et al., 2002; Nover et al., 1983), which would lead to bacterial death (Feng et al., 2000; Matsumura et al., 2002; Melaiye et al., 2005).

The Fe_3O_4 at Ag nanoparticles possessing super paramagnetic and antibacterial properties shows excellent activity against *E. coli, S. epidermis*, and *Bacillus subtilis*. The Fe_3O_4 at Ag nanoparticles were synthesized using the reverse micelle method. The nanoparticles were characterized and detected using UV-visible spectroscopy, TEM and X-ray diffraction (XRD). The antibacterial activity of Fe_3O_4 at Ag nanoparticles was determined with the help of minimum inhibitory concentration (MIC) values. Three bacterial strains *E. coli, B. subtilis* and *S. epidermis* were used by growing the bacterial colonies on Luria Bertani (LB) medium at 37°C up to 10^8-10^9 CFU/ml was reached. 75µL of bacterial suspension was added to 15 ml LB medium containing 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µg/ml Fe_3O_4 at Ag nanoparticles and incubated at 37°C on rotary shaker (200 rpm) for 24 h. The MIC was determined as the lowest concentration when the bacterial growth was inhibited. The MIC values for *E. coli* and *B. subtilis* were found to be >70 µg/ml, while *S. epidermis* showed > 60 µg/ml (Gong et al., 2007).

Silver nanoparticles possess high antimicrobial activity on Gram positive and Gram negative bacteria including multiresistant strains such as methicillin resistant *S. aureus*. The antibacterial activity of silver nanoparticles was found to be size dependent, the nanoparticles of size 25 nm possessed highest antibacterial activity. The nano-

particles were toxic to bacterial cells at lower concentrations of 1.69 µg/ml Ag (Panacek et al., 2006). The use of silver dressings and their role in wound healing was investigated and also reported the role of nanocrystalline silver dressings in wound management (Leaper, 2006). Silver nanoparticles were found to be cytotoxic to *E. coli* and the antibacterial activity of silver nanoparticles was size dependent. Silver nanoparticles mainly in the range of 1-10 nm attach to the surface of cell membrane and drastically disturb its proper function like respiration and permeability (Morones et al., 2005).

Recently, a study revealed the potential cytoprotective activity of Ag NPs toward HIV-1 infected cells. The activity of Ag NPs towards HIV-1 infected Hut/CCR5 cells was investigated using terminal uridyl nucleotide end labeling assay after a three day treatment. The percentage of apoptotic cells was determined as 49, 35, and 19% for vehicle control, 5 and 50 µM Ag, respectively. Ag NPs might inhibit the replication in Hut/CCR5 cells causing HIV associated apoptosis. Size dependent interaction of Ag NPs with HIV-1 virus has also been demonstrated. Ag NPs preferentially bind to gp120 glycoprotein knobs of HIV-1 virus. In the *in vitro* study, it was confirmed that this interaction inhibited the virus from binding with the host cell (Sharma et al., 2009).

SILVER NANOPARTICLES

Different types of nanomaterials like copper, zinc, titanium (Retchkiman-Schabes et al., 2006), magnesium, gold (Gu et al., 2003), alginate (Ahmad et al., 2005) and silver have come up but silver nanoparticles have proved to be most effective as it has good antimicrobial efficacy against bacteria, viruses and other eukaryotic microorganisms (Gong et al., 2007). Silver is widely known as a catalyst for the oxidation of methanol to formaldehyde and ethylene to ethylene oxide (Nagy and Mestl, 1999). Colloidal silver is of particular interest because of distinctive properties, such as good conductivity, chemical stability, catalytic and antibacterial activity (Frattini et al., 2005). Silver is a naturally occurring precious metal, most often as a mineral ore in association with other elements. It has been positioned as the 47th element in the periodic table, having an atomic weight of 107.8 and two natural isotopes 106.90 and 108.90 Ag with abundance 52 and 48%. It has been used in a wide variety of applications as it has some special properties like high electrical and thermal conductivity (Nordberg and Gerhardsson, 1988). Of all the different type of nanoparticle, silver nanoparticles are playing a major role in the field of nanotechnology and nanomedicine.

METAL MICROBE INTERACTIONS

Some microorganisms can survive and grow even at high metal ion concentration due to their resistance to the metal. The mechanisms involve: efflux systems, alteration

Table 1. List of resources synthesized silver nanoparticles.

Resource	Size of nanoparticle (mn)	Reference
Bacteria		
Bacillus cereus	62.8	Silambarasan and Abraham, 2012
Pseudomonas stutzeri AG259	200	Joerger et al., 2000
Klebsiella pneumoniae	52.5	Mokhtari et al., 2009
Bacillus licheniformis	50	Kalimuthu et al., 2008
Staphylococcus aureus	160-180	Nanda and Saravanan, 2009
Fungi		
Aspergillus flavus NCIM 650	8.92 ± 1.61	Vigneshwaran et al., 2007
Aspergillus fumigatus NCIM 902	5-25	Bhainsa and D'Souza, 2006
Aspergillus clavatus AzS-275	10-25	Verma et al., 2010
Fusarium semitectum	35	Basavaraja et al., 2008
Phaenerochaete chrysosporium	50-200	Vigneshwaran et al., 2006
Cladosporium cladosporioides	35	Balaji et al., 2009
Penicillium fellutanum	5-25	Kathiresan et al., 2009
Plant extract		
Emblica officinalis	10-20	Huang et al., 2007
Aloe vera	15.2 ± 4.2	Chandran et al., 2006
Jatropha curcas	10-20	Bar et al., 2009
Henna leaf	39	Kasthuri et al., 2009

of solubility and toxicity via reduction or oxidation, biosorption, bioaccumulation, extracellular complexation or precipitation of metals and lack of specific metal transport systems (Bruins et al., 2000). A little is known about the resistance against the noble metals (Rhodes et al., 1992), although it has been stated that gold can serve as a slow acting drug in rheumatology (Rhodes et al., 1992). Silver is highly toxic to most microbial cells and can be used as a biocide or antimicrobial agent. These metal microbe interactions have important role in several biotechnological applications including the fields of bioremediation, biomineralization, bioleaching and microbial corrosion. Microorganisms considered as potential biofactory for synthesis of metallic nanoparticles such as cadmium sulfide, gold and silver (Sastry et al., 2003).

BIOLOGICAL SYNTHESIS OF SILVER NANOPARTICLES

The use of microorganisms in the synthesis of nanoparticles emerges as an eco-friendly and exciting approach. Silver nanoparticles, like its bulk counterpart, are an effective antimicrobial agent against various pathogenic microorganisms. Though various chemical and biochemical methods are being explored for silver nanoparticles production, microbes are very much effective in this process. Various microbes are known to reduce the metals, most of them are found to be spherical particles as reported earlier (Ahmad et al., 2003a; Sastry et al.,

2003). The resistance conferred by bacteria to silver is determined by the *'sil'* gene in plasmids (Silver, 2003).

Recently, biosynthetic methods employing both biological microorganism such as bacteria (Joerger et al., 2000) and fungus (Shankar et al., 2003) or plants extract (Huang et al., 2007; Gardea-Torresday et al., 2002; Chandran et al., 2006), have emerged as a simple and viable alternative to more complex chemical synthetic procedures to obtain nanomaterials. Biological synthesis of silver nanoparticles using bacteria, fungus and plants extract is listed in Table 1. Extracts from microorganisms may act both as reducing and capping agents in Ag NPs synthesis. The reduction of Ag^+ ions by combinations of biomolecules found in these extracts such as enzymes or proteins, amino acids, polysaccharides, and vitamins (Collera-Zuniga et al., 2005; Jagadeesh et al., 2004) is environmentally benign, yet chemically complex. An extensive volume of literature reports successful Ag NP synthesis using bioorganic compounds. For example, the extract of unicellular green algae *Chlorella vulgaris* was used to synthesize single crystalline Ag nanoplates at room temperature. Proteins in the extract provide dual function of Ag^+ reduction and shape control in the nano-silver synthesis. The carboxyl groups in aspartic and/or glutamine residues and the hydroxyl groups in tyrosine residues of the proteins were suggested to be responsible for the Ag^+ ion reduction. Carrying out the reduction process by a simple bifunctional tripeptide Asp-Asp-Tyr-OMe further identified the involvement of these residues. This synthesis process gave small Ag nanoplates with

low polydispersity in good yield (>55%) (Xie et al., 2007). Most of the researchers have used UV–vis spectroscopy, fourier transform infrared (FTIR) spectroscopy, atomic force microscopy (AFM), scanning electron microscopy (SEM), electron diffraction spectroscopy (EDX) and XRD.

Biosynthesis of silver nanoparticles by bacteria

Bacillus cereus was employed for the extracellular synthesis of silver nanoparticles (Ag NPs) from silver nitrate solution. UV–vis spectroscopy of Ag NPs exhibited peak at 440 nm, which corresponded to the surface plasmon resonance of Ag NPs. FTIR spectroscopy confirmed the presence of protein as the stabilizing agent surrounding the Ag NPs. AFM of Ag NPs showed irregular shape with 62.8 nm in size. Antimicrobial activity of the silver bio-nanoparticles was performed by well diffusion method against S*. aureus*, *Salmonella typhi*, *K. pneumoniae* and *E. coli*. The highest antimicrobial activities recorded were against *S. aureus* followed by *K. pneumoniae* and *S. typhi*, while *E. coli* showed the least activity (Silambarasan and Abraham, 2012).

P. stutzeri AG259 has been reported to synthesize Ag particles (Joerger et al., 2000), which were accumulated within the periplasmic space of bacterial cell of 200 nm. *Lactobacillus*, a common bacterial strain present in the buttermilk, synthesizes of both Au and Ag NPs under standard conditions (Nair and Pradeep, 2002). Rapid synthesis of metallic NPs of Ag using the reduction of aqueous Ag^+ has been achieved in the culture supernatants of *K. pneumoniae*, *E. coli* and *E. cloacae* (Shahverdi et al., 2007).

Aqueous silver ions were reduced to silver nanoparticles when added to the biomass of *B. licheniformis*. In UV-vis spectrum, a strong, broad peak, located between 420 and 430 nm, was observed for silver nanoparticles which were prepared using the culture supernatant. Observation of this peak was assigned to a surface plasmon resonance. EDX analysis also showed a peak in the silver region, confirming the formation of silver nanoparticles. The optical absorption peak is observed approximately at 3 keV, which is typical for the absorption of metallic silver nanocrystallites due to surface plasmon resonance. The XRD pattern of the silver nitrate treated sample was found to correspond to that of silver nanoparticles. The XRD pattern showed four intense peaks in the whole spectrum of 2θ values ranging from 30 to 80. The peaks at 2θ values of 38.25, 46.37, 64.60 and 77.62°, corresponding to 111, 200, 220 and 311 planes for silver, respectively. The full width at half maximum (FWHM) values measured for 111, 200, 220 and 311 planes of reflection were used with the Debye-Scherrer equation to calculate the size of the nanoparticles. The particle sizes obtained from XRD was found to be around 50 nm (Kalishwaralal et al., 2008).

The *Staphylococcus aureus* when subjected to $AgNO_3$,

the reaction started within a few minutes and the colour of the solution turned to yellowish brown indicating the formation of silver nanoparticles. The Ag NPs were characterized by UV-visible spectroscopy. The observation indicated that the reduction of the Ag^+ ions took place extracellularly. The Peak was noted around 420 nm. It was observed from the spectra that the silver surface plasmon resonance band occurs at 420 nm. AFM gave the clear shape and size of the silver nanoparticles produced from the *S. aureus*. The size of the silver nanoparticles solution was found in the range of 160-180 nm which agglomerated and formed distinct nano structures (Nanda and Saravanan, 2009).

Biosynthesis of silver nanoparticles by fungi

Fungi were found to be capable of reducing the metals ions into their corresponding nanometals either intracellularly or extracellularly depending on the position of the reduction enzymes. In other words the nanoparticles were formed extracellular when the cell walls reduction enzymes were responsible for metal ions reduction as well as when the reduction enzymes were secreted extracellular (Mukherjee et al., 2001; Mukherjee et al., 2002). In *Fusarium oxysporum* fungus, the reduction of Ag^+ ions was attributed to an enzymatic process involving NADH dependent reductase (Ahmad et al., 2003b). The white rot fungus, *Phanerochaete chrysosporium*, also reduced Ag^+ ion to form Ag NPs; a protein was suggested to cause the reduction (Vigneshwaran et al., 2006).

Aspergillus flavus (NCIM 650) when challenged with silver nitrate solution accumulates silver nanoparticles on the surface of its cell wall in 72 h. The average size of silver nanoparticles is 8.92 ± 1.61 nm. The FTIR spectrum recorded from the freeze dried powder of silver nanoparticles, formed after 72 h of incubation with the fungus. The amide linkages between amino acid residues in proteins give rise to the well known signatures in the infrared region of the electromagnetic spectrum. The bands seen at 3280 and 2924 cm^{-1} were assigned to the stretching vibrations of primary and secondary amines, respectively; while their corresponding bending vibrations were seen at 1651 and 1548 cm^{-1}, respectively. The two bands observed at 1379 and 1033 cm^{-1} can be assigned to the C–N stretching vibrations of aromatic and aliphatic amines, respectively. The overall observation confirms the presence of protein in the samples of silver nanoparticles. These protein stabilized silver nano particles produced a characteristic emission peak at 553 nm when excited at 420 nm in photoluminescence spectrum (Vigneshwaran et al., 2007).

Extracellular biosynthesis of silver nanoparticles by *A. fumigatus* (NCIM 902) *biomass* was carried out. The fungal biomass after incubation for 72 h with Milli-Q water was separated by filtration. UV-visible spectrum of the aqueous medium containing silver ion showed a peak at

420 nm corresponding to the plasmon absorbance of silver nanoparticles. TEM micrograph showed formation of well dispersed silver nanoparticles in the range of 5 to 25 nm. XRD spectrum of the silver nanoparticles exhibited 2θ values corresponding to the silver nanocrystal (Bhainsa and D'Souza, 2006).

Aspergillus clavatus (AzS-275) an endophytic fungus isolated from sterilized stem tissues of *Azadirachta indica* A. Juss. was challenged with 1 mM AgNO$_3$ solution. The synthesized Ag NPs were found to be extracellular, polydispersed spherical or hexagonal particles ranging from 10 to 25 nm in size. Antimicrobial activity was performed using a disc diffusion method against *Candida albicans*, *P. fluorescens* and *E. coli*. The results showed an average minimum inhibitory concentration of 5.83 mg ml^{-1} and minimum fungicidal concentration of 9.7 mg ml^{-1} against *C. albicans* (Verma et al., 2010).

F. oxysporum biomass with Ag$^+$ ions for 72 h, the biomass has changed from a pale yellow color to a brownish color. FTIR measurements carried out on a drop coated film of the silver nanoparticle-fungus reaction solution showed the presence of three bands at 1650, 1540 and 1450 cm^{-1}. The bands at 1650 and 1540 cm^{-1} are identified as the amide I and II bands and arise due to carbonyl stretch and –N–H stretch vibrations in the amide linkages of the proteins, respectively. The positions of these bands are close to that reported for native proteins. The FTIR results thus indicate that the secondary structure of the proteins is not affected as a consequence of reaction with the Ag$^+$ ions or binding with the silver nanoparticles. The band at 1450 cm^{-1} is assigned to methylene scissoring vibrations from the proteins in the solution. The nanoparticles size was found to be aggregates in the range of 5 to 50 nm (Ahmad et al., 2003b).

The use of fungus *Fusarium semitectum* for the extracellular synthesis of silver nanoparticles has been reported. In UV spectra the silver surface plasmon resonance band occurred at 420nm and this absorption steadily increased in intensity with time of the reaction. FTIR spectroscopic study has confirmed that amino acid and peptides have formed a coat covering the silver nanoparticles to prevent agglomeration. The mean particle diameter of silver nanoparticles was calculated from the XRD pattern using Scherrer's equation. The calculated average particles size of silver nanoparticles was found to be 35 nm (Basavaraja et al., 2008).

Incubation of *P. chrysosporium* mycelium with AgNO$_3$ solution produced silver nanoparticles in 24 h. The synthesized silver nanoparticles absorbed maximum at 470 nm in the visible region. The SEM characterization of the fungus reacted on the Ag$^+$ indicated that the protein might be responsible for the stabilization of silver nanoparticles. This result was further supported by the TEM examination. Though shape variation was noticed, majority of the nanoparticles were found to be of pyramidal shape as seen under TEM. The SEM examination gave the electron micrograph of the silver nanoparticles

on the surface of the mycelia mat with size of approximately from 50 to 200 nm. Photoluminescence spectrum showed a broad emission peak of silver nanoparticles at 423 nm when excited at 350 nm (Vigneshwaran et al., 2006).

The filtrate of the *Cladosporium cladosporioides* supernatant with Ag$^+$ ions, turned from colourless to brown after 24 h of the reaction. The mean particle diameter of Ag NP was calculated from the XRD pattern, the calculated average particle size of the silver was found to be 35 nm. The FTIR showed the presence of two bands at 1640 and 1540 cm^{-1} and are identified as the amide I and amide II and arises due to carbonyl stretch and –N–H stretch vibrations in the amide linkages of the proteins, respectively. IR spectroscopic study has confirmed that the carbonyl group from amino acid residues and peptides of proteins has the strongest ability to bind metal, allowing the proteins to most possibly form a coat covering the metal nanoparticles (that is, capping of Ag NP) to prevent agglomeration of the particles and stabilizing in the medium (Balaji et al., 2009). The extracellular synthesis of silver nanoparticles by a marine fungus *Penicillium fellutanum* has been investigated by Kathiresan et al. (2009). For synthesis of silver nanoparticles AgNO$_3$ 1 mM solution was mixed with 50 ml of cell filtrate and agitated in dark. The presence of silver nanoparticles in reacting mixture was confirmed by absorption peak at 430 nm. The obtained silver nanoparticles were spherical in shape with size ranging from 5 to 25 nm.

Biosynthesis of silver nanoparticles by plant extract

Plants extract from *Ocimum tenuiflorum, Solanum tricobatum, Syzygium cumini, Centella asiatica* and *Citrus sinensis* was used for the synthesis of silver nanoparticles (Ag NPs) from silver nitrate solution. AFM showed the formation of silver nanoparticle with an average size of 28 nm, 26.5 nm, 65 nm, 22.3 nm and 28.4 nm corresponding to *O. tenuiflorum, S. cumini, C. sinensis, S. tricobatum* and *C. asiatica*, respectively. Antimicrobial activity of the silver bionanoparticles was performed by well diffusion method against *S. aureus, P. aeruginosa, E. coli* and *K. pneumoniae*. The highest antimicrobial activity of silver nanoparticles synthesized by *S. tricobatum, O. tenuiflorum* extracts was found against *S. aureus* (30 mm) and *E. coli* (30 mm) respectively (Logeswari et al., 2012).

Synthesis of quasi spherical silver nanoparticles using purified apiin compound, extracted from henna leaf at ambient conditions (Kasthuri et al., 2009). Using green tea, *C. sinensis* extract as reducing and stabilizing agent produced gold nanoparticles and silver nanostructures in aqueous solution at ambient conditions (Nestor et al., 2008). Plant extracts from live alfalfa, the broths of lemongrass, geranium leaves and others have served as green reactants in Ag NP synthesis (Torresdey et al., 2003; Shankar et al., 2003a; Shankar et al., 2005). The

reaction of aqueous $AgNO_3$ with an aqueous extract of leaves of a common ornamental geranium plant, *P. graveolens*, gave Ag NPs after 24 h (Shankar et al., 2003a). A vegetable, *C. annum* L. was also used to synthesize Ag NPs (Li et al., 2007). A rapid reduction of the Ag ions was observed when the $AgNO_3$ solution was contacted with geranium (*P. graveolens*) leaf extract (Shankar et al., 2003b).

Silver nanoparticles ranging from 55 to 80 nm in side and triangular or spherical gold nanoparticles were fabricated using the novel sundried biomass of *C. canphora* leaf (Huang et al., 2007). A simple procedure applying *Aloe vera* leaf extract has been used for gold nanotriangle and spherical silver nanoparticles synthesis. *Aloe vera* extract showed that more spherical particles were formed with increasing amount of added extract (Chandran et al., 2006). Silver nanoparticles were successfully synthesized using the latex of *Jatropha curcas*. The plant, *J. curcas* is commercially important one as biodiesel is extracted from it seeds on industrial scale. Crude latex was obtained by cutting the green stems of *J. curcas* plants (Bar et al., 2009). The bark powder and water extract from *Cynamon zeylanicum* tree were used for silver nanoparticles synthesis (Sathishkumar et al., 2009).

RECENT STUDY ON SILVER DOPED CALCIUM PHOSPHATE NANOPARTICLES

Spherical silver-doped calcium phosphate nanoparticles were synthesized in a co-precipitation route from calcium nitrate/silver nitrate and ammonium phosphate in a continuous process and colloidally stabilized by carboxymethyl cellulose. Nanoparticles with 0.39 wt% silver content and a diameter of about 50 to 60 nm were obtained. The toxic effects toward mammalian and prokaryotic cells were determined by viability tests and determination of the minimal inhibitory and minimal bactericidal concentrations (MIC and MBC). Three mammalian cells lines, that is, human mesenchymal stem cells (hMSC) and blood peripheral mononuclear cells (PBMC, monocytes and T-lymphocytes), and two prokaryotic strains, that is, *E. coli* and *S. aureus* were used. Silver-doped calcium phosphate nanoparticles and silver acetate showed similar effect toward mammalian and prokaryotic cells with toxic silver concentrations in the range of 1-3 µg mL^{-1} (Peetsch et al., 2013).

MECHANISM OF SILVER NANOPARTICLES BIOSYNTHESIS

Studying the synthesis of Ag NPs with isolated or purified bioorganics may give better insight into system mechanism. Glutathione (γ-Glu-Cys-Gly-) as a reducing or capping agent can produce water soluble and size tunable Ag NPs that easily bind to model protein (bovine serum albumin) attractive for medical applications. Tryptophan

residues of synthetic oligopeptides at the C-terminus were identified as reducing agents giving Ag NPs (Si and Mandal, 2007). Furthermore, Ag NPs were successfully synthesized by vitamin E in the Langmuir-Blodgett technique by biosurfactants such as sophorolipids and by L-valine based oligopeptides with chemical structures, Z-(L-Val)$_3$-OMe and Z-(L-Val)$_2$-L-Cys(S-Bzl)-OMe. The sulfur content in the Z-(L-Val)$_2$-L-Cys(S-Bzl)-OMe controls the shape and size of Ag NPs, which suggests the interaction between the Ag^+ ion and the thioether moiety of the peptide (Mantion et al., 2008).

The enzyme involved in the synthesis of nanoparticles may be the nitrate reductase present in *Bacillus licheniformis* (Kalimuthu et al., 2008). This enzyme is induced by nitrate ions and reduces silver ions to metallic silver. The possible mechanism that may involve in the reduction of silver ions is the electron shuttle enzymatic metal reduction process (Figure 1), earlier proposed for gold nanoparticles (He et al., 2007). The reduction seems to be initiated by electron transfer from the NADH by NADH-dependent reductase as electron carrier and this enzyme is an important factor in the biosynthesis of metal nanoparticles. *B. licheniformis* is known to secrete the cofactor NADH and NADH-dependent enzymes, especially nitrate reductase, that might be responsible for the bioreduction of Ag^+ to Ag^0 and the subsequent formation of silver nanoparticles.

MECHANISM OF ACTION OF SILVER NANOPARTICLES

The bactericidal effect of silver nanoparticles is size dependent (Morones et al., 2005). The antimicrobial efficacy of the nanoparticle depend on the shapes of the nanoparticles also, this can be confirmed by studying the inhibition of bacterial growth by differentially shaped nanoparticles (Morones et al., 2005). The truncated traingular nanoparticles show bacterial inhibition with silver content of 1 µg. While, in case of spherical nanoparticles total silver content of 12.5 µg is needed.

The rod shaped particles need a total of 50 to 100 µg of silver content (Pal et al., 2007). Thus, the silver nanoparticles with different shapes have different effects on bacterial cell. The mechanism of the bactericidal effect of silver and Ag NPs remains understood. Several studies propose that Ag NPs may attach to the surface of the cell membrane disturbing permeability and respiration functions of the cell. Smaller Ag NPs having the large surface area available for interaction would give more bactericidal effect than the larger Ag NPs (Kvitek et al., 2008). It is also possible that Ag NPs not only interact with the surface of membrane, but can also penetrate inside the bacteria (Morones et al., 2005).

The silver nanoparticles showed efficient antimicrobial property compared to other salts due to their extremely large surface area, which provides better contact with microorganisms. The nanoparticles get attached to the
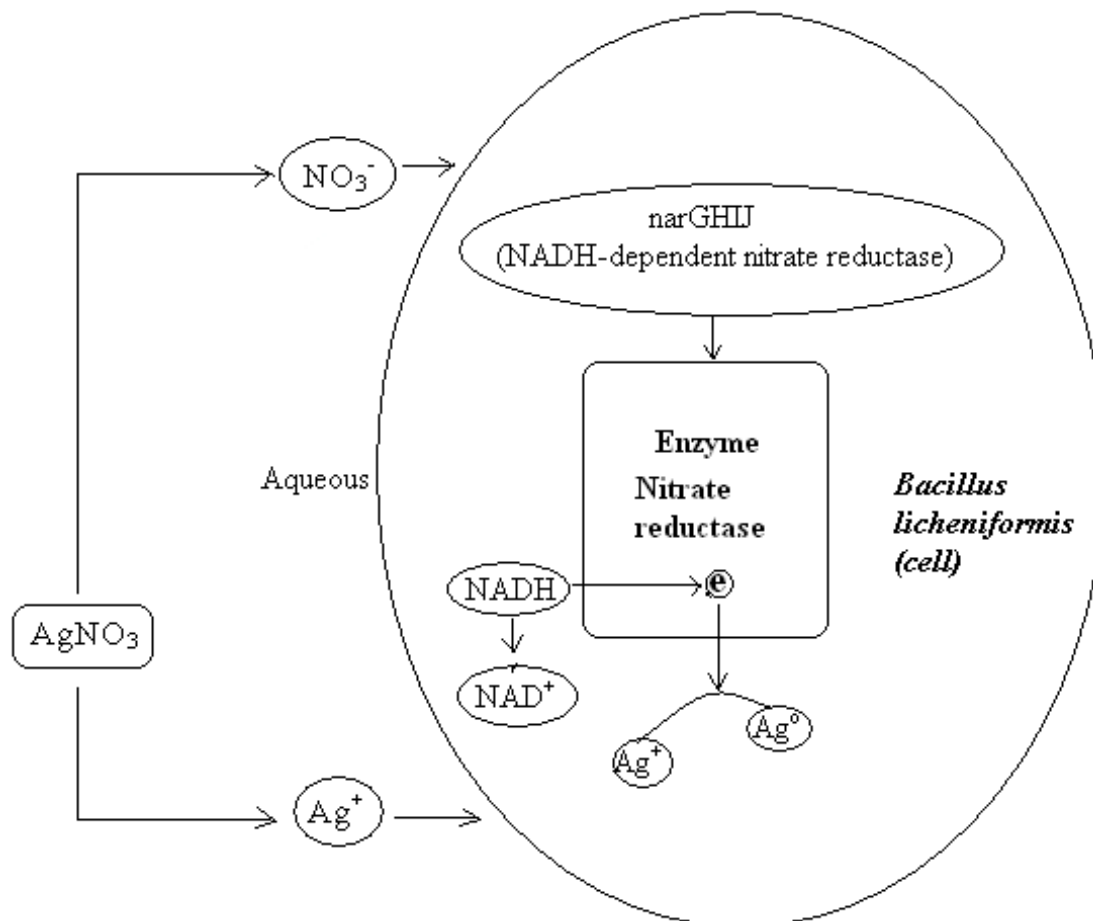
Figure 1. The possible mechanism for the biosynthesis of silver nanoparticles by *B. licheniformis*, involving NADH-dependent nitrate reductase enzyme that may convert Ag^+ to Ag^0 through electron shuttle enzymatic metal reduction process (Kalimuthu et al., 2008).

cell membrane and also penetrate inside the bacteria. The bacterial membrane contains sulfur containing proteins and the silver nanoparticles interact with these proteins in the cell, as well as with the phosphorus containing compounds like DNA. When silver nanoparticles enter the bacterial cell it forms a low molecular weight region in the center of the bacteria to which the bacteria conglomerates thus, protecting the DNA from the silver ions. The nanoparticles preferably attack the respiratory chain, cell division finally leading to cell death. The nanoparticles release silver ions in the bacterial cells, which enhance their bactericidal activity (Morones et al., 2005; Feng et al., 2000; Sondi and Salopek-Sondi, 2007; Song et al., 2006). Silver is known for its antimicrobial properties and has been used for years in the medical field for antimicrobial applications and even shown to prevent HIV binding to host cells (Russell and Hugo, 1994). Additionally, silver has been used in water and air filtration to eliminate microorganisms (Chou et al., 2005; Jin et al., 2007). Silver ions play a crucial role in the antibacterial activity of silver zeolite. Possible action of silver zeolite might be due to the intake of silver ions by bacterial cells

when they come in contact with silver zeolite, which inhibits their cellular functions and damages the cell. Secondly, it can be due to the generation of reactive oxygen molecules, which inhibit the respiration (Matsumura et al., 2003).

Low concentrations of Ag^+ ion result in massive proton leakage through the *V. cholera* membrane. This proton leak might be happening from either any Ag^+ modified membrane protein or any Ag^+ modified phospholipids bilayer. The phenomenon causes deenergization of the membrane and consequently cell death (Dibrov et al., 2002). Importantly, the determined effective concentration of Ag NPs was at nanomolar levels while Ag^+ ions were effective at micromolar levels (Lok et al., 2006). Ag NPs thus seem to be more efficient than Ag^+ ions in performing antimicrobial activities. Picomolar levels of Ag NPs, on the other hand, have been used as nanoprobes in membrane penetration studies and did not create significant toxicity to the cells (Xu et al., 2004).

The Ag NPs obtained in the reduction of the Ag $(NH_3)_2^+$ complex cation by monosaccharides and disaccharides with narrow size distribution were tested as antimicrobial

agents. Ag NPs synthesized using disaccharides such as maltose and lactose have a higher antibacterial activity than those synthesized using monosaccharides such as glucose and galactose. The sizes of the colloidal Ag particles were smaller for disaccharide than monosaccharide and thus may be responsible for the observed antibacterial activity. The 25 nm sized Ag NPs synthesized via reduction by maltose showed the highest activity and were comparable to the effects of ionic silver in certain bacteria strains. Galactose had the largest Ag NPs particles (50 nm), and gave the lowest antimicrobial effect (Panacek et al., 2006).

APPLICATIONS

Silver has been known to possess strong antimicrobial properties both in its metallic and nanoparticle forms hence; it has found major application in the field of medicine.

1. Silver in ion or metallic can be exploited in medicine for burn treatment, dental materials, water treatment, sunscreen lotions, etc. (Duran et al., 2007).
2. The silver nanoparticles were also used for impregnation of polymeric medical devices to increase their antibacterial activity. Silver impregnated medical devices like surgical masks and implantable devices showed significant antimicrobial efficiency (Furno et al., 2004).
3. Researchers have also recommended the use of silver and copper ions as superior disinfectants for wastewater generated from hospitals containing infectious microorganisms (Lin et al., 1996).
4. Silver nanoparticles can be used for the production of antimicrobial paints based on vegetable oil. The Ag NP embedded drying oil is an excellent coating material and can be used to coat several kinds of surface such as wood, glass, polypropylene, poly(methyl methacrylate), polystyrene and building walls made of different materials. As MNPs are homogeneously dispersed in vegetable based drying oil, the adhesion properties of Ag NP embedded paints were tested by coating them on different substrates such as glass and polymers (Kumar et al., 2008).
5. Silver has been used extensively for the treatment of burns (George et al., 1997) with AgSD incorporated into bandages for use in large open wounds (Wright et al., 1999).
6. Silver nanoparticles can be coated on common polyurethane (PU) foams by overnight exposure of the foams to nanoparticle solutions. Repeated washing and air drying yields uniformly coated PU foam, which can be used as a drinking water filter where bacterial contamina-tion of the surface water is a health risk. Nanoparticles are stable on the foam and are not washed away by water. The nanoparticle binding is due to its interaction with the nitrogen atom of the PU (Jain and Pradeep, 2005).
7. Dressings have a part to play in the management of

wounds; whether they are sutured or open, usually chronic wounds of many aetiologies which are healing by secondary intention. Nanocrystalline technology appears to give the highest, sustained release of silver to a wound without clear risk of toxicity (Leaper, 2006).
8. Silver zeolite is used in food preservation, disinfection and decontamination of products (Matsuura et al., 1997; Nikawa et al., 1997).

CONCLUSION

The biological synthesized silver nanoparticles could be of immense use in medical textiles for their efficient antimicrobial function. The antimicrobial efficacy of the nanoparticle depend on the shapes of the nanoparticles also, this can be confirmed by studying the inhibition of bacterial growth by differentially shaped nanoparticles. The silver nanoparticles with their unique chemical and physical properties are proving as an alternative for the development of new antibacterial agents. The silver nanoparticles have also found diverse applications in the form of wound dressings; coatings for medical devices, silver nanoparticles impregnated textile fabrics, etc. Further researches are needed to synthesize the silver nanoparticles by using different microbes and also to study the biochemical and molecular mechanism of nanoparticles formation by the cell filtrate in order to achieve better control over size and polydispersity of the nanoparticles.

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