Biosynthesis and characterization of silver nanoparticles from *Penicillium notatum* and their application to improve efficiency of antibiotics

Shareefraza J. Ukkund*, Raghavendra M. J., Yashawantha K. Marigowda, Abhinaya N., Prasad Puthyillam*

1Department of Nano-Technology, Srinivas Institute of Technology, Mangalore-574143- India
2Department of Mechanical Engineering, Srinivas Institute of Technology, Mangalore-574143- India
3Department of Nano-Biotechnology, Srinivas Centre for Nano Science and Technology, Srinivas Group of Institutions, Mangalore-574143-India

Email: shareef@sitmng.ac.in Ph: +91 8884975771

**Abstract:** Nanomaterials can be synthesized by physical, chemical and biological methods. The biological method of synthesis of silver nanoparticles have more advantage and gives high purity. Silver nanoparticles are having antibacterial properties and can be synthesized by any microbes and plants with minimal effort. The present study includes the synthesis of silver nanoparticles from penicillium notatum which is well known producer of penicillium antibiotic. The produced nanoparticles of silver are then characterized by UV-vis spectrophotometer for primary confirmation, XRD for structural analysis, FTIR for functional group, SEM, TEM and AFM for morphological analysis. The SEM and AFM results revealed that the size of silver nanoparticles were of range 55-65 nm. TEM result reveals the spherical shape of silver nanoparticles. Optimization of silver nanoparticles method is also performed to understand the maximum yield at specific substrate concentration, pH and salt concentration. The silver nanoparticles are then conjugated with six antibiotics for antibacterial activity by zone of inhibition method. The result indicated that silver nanoparticles increased the efficiency of streptomycin 2 fold and erythromycin by 3 fold.

**Keywords:** Silver nanoparticles; Antibacterial activity; *penicillium chrysogenum (penicillium notatum)*; UV-Vis-spectrophotometer; XRD; SEM; AFM.

1. Introduction

Nanotechnology is an interdisciplinary, it involves manipulating and monitoring atomic and molecular level to fabricate and develop new materials. The nanomaterials range falls under 1-100 nm meter range any materials falls under this range will have different electrical, optical and mechanical etc., properties compare to bulk materials. The most important element of Nanotechnology is the fact that it deals with things which cannot be seen with naked eyes. Nano scale science is the study of objects at a very small scale, approximately 1 to 100 nanometres (nm). A grain of sand is 1 million nm or 1mm wide. Building the machines at the molecular level is called as nanotechnology. The developing field of nanoscience and nanotechnology are becoming more and more popular every day [1].

Nanotechnology finds its significant importance in each and every field including electrical, civil, renewable energy, environmental, agriculture, food industry, chemical industry, textile, and medical,
automobile, aeronautical and information like quantum computation etc. The nanomaterials of different morphology will have different importance based on the properties which can be used in above mentioned applications. These nanomaterials can be synthesised, fabricated and surface is modified to design nanobased devices or tools which will bring the revolutionary change in future [2].

Silver nanoparticles (AgNPs) are the kind of nanoparticles that is in the range between that of 1 nm and 100 nm it has properties that includes electrical, optical and thermal and are present in between the ranges from photovoltaic’s to biological and sensors, for the purpose of excellent conductivity and sensitivity silver nanoparticles are used as conductive inks, pastes and fillers one of the major application of silver nanoparticles is in case of textile industries, antimicrobial coatings, wound dressing and biological devices were it helps to protect from bacteria. Silver nanoparticles are good in absorbing and scattering light and, compared to other components, many pigments and dyes it consists of colour related on shape and the size of the silver particle [3].

Silver nanoparticles have excellent antibacterial, antifungal and antiviral activities. Thus these nanoparticles can be used in scaffolds for bones regeneration, biomedical devices and dental composites [4]. Silver nanoparticles are also used in water purifiers as it is harmful to bacteria and fungus. The silver nanoparticles can be synthesised by microbes. The fungal synthesis finds very significant morphological characteristics by fungus Trichoderma Reesei. The silver nanoparticles also got significant importance in air filters, food industry as packing materials or containers and textile industry for antibacterial clothes etc. Thus silver nanoparticles are most widely synthesised by researches for their attractive properties. Nanomaterials has excellent surface to volume ratio which makes them significantly active since the surface atoms are more compare to inner atoms so does silver nanoparticles[5-6].

Silver nanoparticles are nowadays finding significant impact in biomedical devices and implants including heart valves, masks, wound dressing and bandages. The comparative study was carried out of silver nanoparticles with gold nanoparticles where in silver showed much excellent antibacterial activity than gold nanoparticles [7].

The silver nanoparticles was synthesised by fungi fuzarium oxysporium and tested against the antibiotics to improve their antibacterial activity. The silver nanoparticles were of average 75 nm in size. The efficiency of activity of antibiotics is increased by several folds by conjugating the AgNPs with the different types of antibiotics by using zone of inhibition method and erythromycin showed 2 fold increments [8]. Most recent years many studies have been done on silver nanoparticles for their use in medical fields and their safety issues but there are very fewer studies have been done on silver nanoparticles usage as antiseptic agent in fabrics [9].

The emerging research in this field of interest producing the nanostructures by biological methods; that is by using microbes as an alternative approach for the chemical and physical methods of synthesis. The nanostructures can be synthesised by bacterias [10, 11], fungi [8] and plants gain much attention as they are simple and feasible. The nanoparticles has very high surface to volume ratio which means the surface atoms are more compare to the inner atoms which are responsible for the reactivity which makes nanostructures with excellent electric, optical and catalytic properties [12-13].

Silver has antibacterial property which makes it as very influential substance due to its action against mammalian tissue where it can be used as antiseptic agent [8, 14]. Silver results cytotoxic effects against microbes thus it can be utilised as antimicrobial agent [8].

The catalytic characteristics of metal nanostructures have got significant importance in biotechnological fields for targeted drug delivery, bionanosensors, antibacterial and antifungal agents [8, 15 &16]. Silver nanoparticles play vital role in medical fields as it is toxic to the microbial cell wall [8, 17 & 18].
Penicillium notatum is the species of fungus is common in temperate and subtropical regions and can be found on salted food products commonly [19]. The fungi are widely found in indoor environments, especially in damp or water damaged buildings. It is also known as penicillium chrysogenum [20].

2. Experimental Procedure

2.1 Collection of Sample

The sample is collected from the old building near Varada river shore situated in Haveri city, Karnataka, India. Aseptic method was used to reduce contamination during collection of the sample. 5 various samples were collected from the in 1 square foot area by Random Sample technique.

2.2 Preparation of specimen

The soil collected was diluted with double distilled H\textsubscript{2}O to prepare solution of specimen. The specimen was diluted serially until 10\textsuperscript{-6} dilution.

2.3 Serial dilution

The sterilised plates are prepared. Initially 9ml of buffer is added into 5 test tubes with aseptic method. The 1ml of water was added into 10\textsuperscript{-1} tube. The solution was mixed for some time. The dilution method is continued for sterilised tubes of 10\textsuperscript{-2} until 10\textsuperscript{-6}. After every dilution, the sample was poured into Nutrient agar plate by spread plate technique.

2.4 Spread Plate method

The 6 samples (10\textsuperscript{-1} to 10\textsuperscript{-6}) from serial dilution are transferred to the petriplates which contains the nutrient agar media for microbial growth.

2.4.1 Media preparation

Media used here is Nutrient agar. 200ml of Nutrient agar is prepared and then the solution was autoclaved to remove microbes and other contaminants.

Procedure: The 1ml of diluted sample was poured on the agar plate and made sure that it spreads evenly on the surface of agar plate. The culture was then kept for incubation for colony growth.

2.4.2 Pure culture

After incubation of culture the various kinds of colonies were observed, to get one pure culture we had selected only one colour type colony and inoculated. Then the plates were incubated at room temperature for 4 days until colonies appear. The colonies of colour pink, green, and yellow are observed, which gives confirmation of *penicillium chrysogenum*. The single colony white colour was selected to prepare liquid broth.

2.4.3 Culture of penicillium notatum

A pure culture of obtained sps is then subjected to microbial analysis and the present specific fungi “chrysogenum”, which was used for the extra cellular biosynthesis of silver nanoparticles.

2.5 Preparation of Biomass and Liquid broth

The biomass is produced by culturing the penicillium notatum aerobically. This biomass was used to prepare silver nanoparticles by treating with the silver nitrate solution. 200 ml of liquid broth was prepared and one of the pure cultured colonies was inoculated and kept for incubation at room temperature for 7 days.
2.6 Natural Biosynthesis of Ag nanostructure by AgNO₃

After the growth of the fungus in the flask containing liquid broth was then filtered using Whatman channel paper No.1, to get homogenous solution. This of pH the solution reported to be 6.5. The 1mM (0.017g/100ml) of silver nitrate is prepared and then 50 ml is added to the 50 ml of filtrate (nutrient broth solution containing suspended microbes). The reaction was conducted under dark. The control without silver particle was used against test sample. The biosynthesis of silver nanoparticles was monitored at different time intervals at 12, 24, 48 and 72 hours and the absorbance was calculated at wavelengths range 200 to 800nm.

2.7 Microwave assisted synthesis

The nanoparticles can also be synthesized by using microwave method to overcome time consuming for production of silver nanoparticles. The microwave method employs the heating of the microbial solution (Nutrient broth) for few seconds before adding silver nitrate. After heat treatment the microbes release their metabolic enzymes very rapidly which play as reduction agents for the reducing silver nitrate to silver nanoparticles.

The 50ml of microbial is subjected to microwave of 600 watt for 30 seconds and then the solution is the treated with the 50ml of 1mM silver nitrate (2:1 ratio- 100ml broth: 50ml silver nitrate). Again the solution is subjected to microwave over for few minutes at 600 watt.

2.8 Optimization of synthesised silver nanoparticles

The solution having silver nanoparticles were tested for their maximum production at different parameters such as concentration of AgNO₃, pH and salt concentration and synthesis is optimised. 300 µl of solution was removed to quantify the absorbance at a wavelength of 430 nm.

2.9 Characterization and Examination

As soon as the synthesized the synthesized AgNPs were characterized by UV-vis spectrophotometer for primary confirmation of synthesized silver nanoparticles. The solid nanoparticles are obtained by centrifugation of solution. The smear is prepared with the pellet obtained. Then AgNPs were characterized by XRD for structural analysis, FTIR for functional groups, SEM, TEM and AFM for morphological studies.

2.10 Investigation of Antibacterial activity of AgNPs

The synthesized silver nanoparticles are conjugated with the four antibiotics and monitored to check for their antibacterial activity by zone of inhibition test. The antibiotics used are Fusidic acid (Fus), Clavulanic acid (Cla), Erythromycin (Ery), Streptomycin (Stp), Daptomycin (Dap) and Spiramycin (Spi). The both gram positive and gram negative microbes are cultured on Muller Hinton agar media and 10 µl of silver nanoparticles solution is added along with four antibacterial discs. The culture is then kept for incubation for 48 hours and then the antibacterial activity of the four antibiotics are evaluated by measuring the diameter of the colonies that is zone of inhibition by antibiotics using zone of inhibition test.

3. Results and Discussion

3.1 Primary confirmation

Visual confirmation: The very first characterization of synthesized silver nanostructures done by just observing the colour change from yellow to brown. This can be called as a primary confirmation of formation of AgNPs. The 1 mM AgNO₃ is added to the test sample and the solution was incubated for
42 hours the solution turned to brownish in colour in natural biosynthesis and wherein microwave synthesis the brown colour was obtained in 5 minutes.

3.2 UV-Spectrophotometer

The synthesized silver nanoparticles are transferred to covet and subjected to UV scan from 200 to 800 nm wavelength period by using Double beam UV-visible Spectrophotometer. The readings of synthesized silver nanoparticles are taken at regular time intervals 12, 24, 48, and 72 hours, the highest peak we got it for 48 hours and further the absorbance started decreasing this might be because of the inactivation of nitrate reductase enzymes or reducing agents present in the penicillium notatum. The UV spectrophotometer readings are recorded (Figure 1). The highest peak was found at maximum absorbance 2.6 at 48 hours at 440 nm.

![UV Spectrophotometer readings of biosynthesis of silver nanoparticles](image)

**Figure 1.** UV- spectral recordings at 12, 24, 48 and 72 hours

The above data implies that nitrate reductase enzyme unconfined into cell filtrate solution after the electrostatic interaction with the silver nitrate. Then the silver ions are reduced to AgNPs by the enzymatic reduction thus producing AgNPs extracellularly.

3.3 Optimization of AgNPs

3.3.1 Influence of AgNO₃ concentration

The synthesis of AgNPs was monitored with liquid broth 0.5, 1.0, 1.5, 2.0, 2.5 mM of silver nitrate and incubated. The highest yield was found at 1.5 mM AgNO₃ in Figure 2.
3.3.2: Influence of pH

The synthesis of AgNPs was monitored and the highest yield was found at pH 6.5 (Figure 3). It was also reported that the significant formation of silver nanoparticles was found at around pH 6.5 as the nanostructures absorbed maximum light at this stage. The Shareef et al. [5] studied on the same conditions where they reported that the maximum production was found at pH 6 which was bit acidic for applications related to clinical studies. As adapted from their study reason for the reduction in peak as it moves for neutral pH might be because of deactivation of enzymes which are responsible for the reduction of silver nitrate to silver nanoparticles.

3.3.3: Effect of salt concentration

The formation of nanoparticles are manipulated by varying salt concentration (0.1, 0.2, 0.3, 0.4, 0.5% NaCl) (Figure 4). The significant formation of nanoparticles was found at NaCl concentration of 0.2%. The salt concentration at 0.2% results the stable and excellent formation of AgNPs.
3.4 FTIR analysis

FTIR results confirms of presence of silver nanoparticles along with microbial suspensions with proteins and other derivatives (Figure 5). The interaction between the cell filtrate and AgNO₃ was also confirmed by using FTIR peaks in the range of 4000-500 cm⁻¹ wave number.

3.5 XRD analysis

The XRD patterns (Figure 6) reveal that the synthesized silver nanoparticles were of size range 50-60 nm by using Debye-Scherrer's formula. The produced XRD data were matching with standard data for silver (JCPDS 87 – 0720).
3.6 SEM Analysis

The synthesised silver nanoparticles are then subjected to centrifuge to obtain a pellet to obtain SEM results (Figure 7 a).

3.7 AFM analysis

The Atomic Force Microscope image reveals the surface of silver nanoparticles. The image clearly shows silver nanoparticles of size range 55-65 nm. We can even see the spherical shape of silver nanoparticles from the AFM image (Figure 7 b).

3.8 TEM analysis

The Transmission Electron Microscope image reveals that the synthesized silver nanoparticles are spherical in nature (Figure 7 c).

3.9 Zone of inhibition

The synergistic antibacterial movement of silver nanoparticles with distinctive anti-infection agents was studied. The zone of inhibition of silver nanoparticles is studied against gram positive and gram negative were examined independently. The results are organized in Table 1.

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**Figure 6.** XRD pattern of silver nanoparticles

**Figure 7 a.** The SEM image of silver nanoparticles of size 55-65 nm

**Figure 7 b.** The AFM image of silver nanoparticles of size 55-65 nm

**Figure 7 c.** The TEM image of spherical silver nanoparticles
Table 1. Zone of inhibition for different antibiotics against the test organisms

| Antibiotics | Fusidic acid (10µg/disc) | Erythromycin (10µg/disc) |
|-------------|--------------------------|--------------------------|
|             | Fus (zone in mm) | Fus+AgNP (zone in mm) | Fold increase (in %) | Ery (zone in mm) | Ery+AgNP (zone in mm) | Fold increase (in %) |
| S.aureas    | 6                    | 12                      | 100                   | 0                  | 0                      | 0                      |
| E coli      | 0                    | 0                       | 0                     | 2                  | 6                      | 200                    |

| Antibiotics | Clavulanic acid (10µg/disc) | Daptomycin (10µg/disc) |
|-------------|-------------------------------|------------------------|
|             | Cla (zone in mm) | Cla +AgNP (zone in mm) | Fold increase (in %) | Dap (zone in mm) | Dap+AgNP (zone in mm) | Fold increase (in %) |
| S.aureas    | 13                       | 16                     | 63.07                 | 18                | 20                     | 11.11                  |
| E coli      | 0                        | 0                      | 0                     | 0                 | 0                      | 0                      |

| Antibiotics | Streptomycin (10µg/disc) | Spiramycin (10µg/disc) |
|-------------|--------------------------|------------------------|
|             | Stp (zone in mm) | Stp+AgNP (zone in mm) | Fold increase (in %) | Spi (zone in mm) | Spi+AgNP (zone in mm) | Fold increase (in %) |
| S.aureas    | 0                        | 0                      | 0                     | 0                 | 0                      | 0                      |
| E coli      | 4                        | 12                     | 200                   | 8                 | 12                     | 50                     |

The results showed that there was exactly 2 fold increment in the efficiency of antibiotics. The antibiotic Fusidic acid showed 1 (100%) fold improvements in the efficiency where as Streptomycin showed 2 (200%) and Erythromycin showed (300%) fold improvements, which shows the significant effective antimicrobial property of silver nanoparticles. Other antibiotics like Clavulanic acid, Spiramycin and Daptomycin also showed brilliant improvement in their efficiency by 63.07 %, 50% and 11.11% respectively.

These results can be compared with other research groups who conducted study on antimicrobial property of silver nanoparticles to improve the efficiency of antibiotics and present work results shows that more work has been done in the area. As compared to the study done by Guangquan et. al. [20] which had an increase of 70-80% efficiency in the zone of inhibition test, ours had 200% increase (threefold increase) for the antibiotic Erythromycin. Also we can compare with work done by Shareef et. al. [8] had an increase of 100% efficiency in the zone of inhibition test; ours had 200% improvement in efficiency of Erythromycin.

4. Conclusion

Penicillium notatum was utilised for biosynthesis of silver nanoparticles. The biological method has got many advantages over chemical and physical methods as it is ecofriendly and economical. The biosynthesised nanoparticles can be used as bactericidal, medical, material science and other applications makes this method significantly used high yield green synthesis of silver nanoparticles and other inorganic nanomaterials. The Penicillium notatum is observed to have a maximum production of nanoparticles 48 hours by natural biosynthesis (48 Hours) is reduced by the microwave assisted biosynthesis method (20 Minutes). The fuazarium oxysporium is observed to have a significant formation of nanoparticles at pH 6.5, AgNO₃ concentration of 1.5 mM silver nitrate and at a salt concentration of 0.2%. The average size of the nanoparticles found to be 55-65 nm by SEM,
AFM and TEM analysis and XRD peaks showed that the structure of nanoparticles is FCC in nature. FTIR pattern reveals presence of silver nanoparticles with nitrate reductase enzymes on the surface of AgNPs. Zone of inhibition test proved that there is an increase in stability of nanoparticles. Antibacterial activity of silver nanoparticles was checked against six antibiotics where in Erythromycin (300%) and Streptomycin (200%) showed highest fold increase followed by Fusidic acid (100%), Clavulanic acid (63.07%) and Spiramycin (50%).

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