Int. J.Nano Dimens. 4(1): 77-83, Summer 2013
ISSN: 2008-8868

Contents list available at **IJND**

International Journal of Nano Dimension

Journal homepage: *www.IJND.ir*

Extracellular synthesis, characterization and antibacterial activity of Silver nanoparticles by *Actinomycetes* isolative

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Received 11 August 2012
Accepted 12 November 2012

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ABSTRACT

The development of the eco friendly procedures makes nanoparticles as the rapidly growing field of nanotechnology. Amongst, the silver nanoparticles have become prominent in the field of medicine to their peculiar antimicrobial properties. In the present study we suggest an eco friendly procedure of extracellular synthesis of silver nanoparticles with an average sizes of 5-50nm using an *Actinomycete* isolated from mangrove soil. The silver nanoparticles were characterized with UV-Visible spectrophotometer, FTIR and TEM analysis. The synthesized silver nanoparticles showed an excellent antibacterial property on multidrug resistance gram positive and gram negative bacterial strains.

Keywords: *Actinomycetes spp; Silver nanoparticles; Characterization; Antibacterial activity; Extracellular synthesis; TEM.*

INTRODUCTION

Nanotechnology is the widely aspiring field of science which is producing novel applicative materials and technologies where conventional methods become obsolete [1]. Nanoparticles of metal, semiconductor, ceramic etc, are preparing by various physical and chemical methods [2, 3, and 4]. Now days it is necessary to develop clean, non-toxic and environmental friendly procedures of nanoparticle synthesis. The inspiration taken from the nature has favored the use of microbes in the reduction of toxic metal ions into stable metals (5). Novel metal nanoparticles like silver, gold were synthesized extensively by employing various bacterial and fungal strains. For instance the bacterial strain, *Pseudomonas stutzeri* [6] from silver mines had produced the silver nanoparticles.

Similarly, silver nanoparticles are being extensively synthesized by various fungi either intracellular or extracellularly. Sastry *et al.*, [7] produced silver nanoparticles within the cell walls of *Verticillium sps* and Vigneshwaran *et al.*,[8] from *Aspergillus flavus*.other workers used a variety of fungal strains like *Fusarium oxysporum*,[9] *Fusarium semitectum*,[10] *Aspergillus fumigatus* [11] for synthesis of nanoparticles. Silver nanoparticles have several important applications like intercalation materials for electrical batteries [12], optical receptors [13], polarizing filters, and catalysts in chemical reactions, biolabelling [14], sensors [15], and bioactive materials [16], Silver nanoparticles are also being used as an enhanced substrate in surface enhanced Raman spectroscopy (SERS) for enzyme immunoassay [17]. The antimicrobial activity of silver ion Ag^+ has been exploited for a longtime in the biomedical field [18]. The silver nanoparticles having the size 5nm and below are interacting with the gp120 protein of HIV-I Virus inhibits the propagation of the virus [19]. The biosorption of heavy metal ions by fungal strain, *A.niger* was reported [5, 20] but the extraction and characterization of the biosorbed metal ions were not studied properly. Considering the applicative aspect of the silver nanoparticles in various fields of commercialization, in this paper we suggest an ecofriendly procedures for synthesis of silver nanoparticles using *Actinomycete* isolate from mangrove soil. The silver nanoparticles were synthesized extracellularly and characterized with UV-Vis, FTIR and TEM analysis. The synthesized nanoparticles were tested for their antibacterial activity on both gram positive and gram negative bacterial strains which cause the diseases in human beings.

EXPERIMENTAL

Sample collection

The Mangrove soil was collected from coastal regions of Andhra Pradesh, India. Soil samples are taken from 3 to 4cm depth with help of sterile spatula, in sterile plastic bags. The samples were brought to laboratory for further studies.

Isolation of Actinomycetes

Actinomycetes culture was isolated by soil serial dilution technique. Distinct *Actinomycetes* colonies were screened and further purified by sub culturing medium and finally maintained on the same slants further studies.

Biosynthesis of silver nanoparticles from Actinomycetes isolate

To prepare the biomass of *Actinomycetes* culture, it was grown aerobically in a liquid medium and the flasks were inoculated with *actinomycetes* and incubated on orbital shaker at 25°C and agitated at 150 rpm. The *Actinomycetes* biomass was harvested after 72 h of growth by sieving through a plastic sieve, followed by extensive washing with distilled water to remove any remains of medium. Typically 10 g of biomass of *actinomycetes* (fresh weight) was brought in contact with 100 ml of Milli-Q deionized water for 72 h at 25°C in an Erlenmeyer flask and agitated in the same condition as described earlier. After the incubation, the cell filtrate was obtained by passing it through Whatman filter paper No. 1. For synthesis of silver nanoparticles, 1mM $AgNO_3$ was mixed with 50 ml of cell filtrate in a 250 ml Erlenmeyer flask and agitated at 25°C in dark. Control (without the silver ions, only biomass) was also run along with the experimental flask.

Characterization of silver nanoparticles

- **UV-Visible absorption spectral analysis**

The absorption spectrum of silver nanoparticles was obtained with the JASCOV-530 (Japan) UV-VISIBLE spectrophotometer. For this analysis 3ml of the filtrate sample was withdrawn from the flask at regular time intervals of 24hr and recorded within the wavelength range of 200-800nm.

- **FTIR and TEM analysis**

The *Actinomycetes* filtrate containing silver nanoparticles was analyzed with the Perkin Elmer Fourier Transform Infrared Spectrometer. The spectrum was recorded in AT mode with resolution 0.2 in the wavelength range of 40-400nm.One 1ml sample aliquots was withdrawn at different time intervals starting from 1 to 48th hours , the absorbance was measured by using UV–

visible spectrophotometer (JASCO V-530 –Japan) with wavelength scanning from 200-800nm. On completion of the reaction of the silver ions with the *Actinomycetes* biomass after 72 h of incubation, cell filtrates containing nanoparticles were subjected to Fourier Transform Infrared Spectroscopy (FTIR) studies, which were carried out in a Shimadzu FTIR-8201 PC instrument in the diffuse reflectance mode at a resolution of 4 cm_1. In order to obtain good signal / noise ratio, 512 scans were recorded. The silver nanoparticles size was determined with Transmission Electron Microscope.

- **Nitrate reductase assay**

The Nitrate reductase assay was performed [21]. The reagents used were: assay medium: 30mM KNO_3 and 5% propanol in 0.1M phosphate buffer, pH 7.5; nitrite solution: 25µM $NaNO_2$ (Nitrite) solution; nitrite assay reagents: sulfanilamide solution: 1% (w/v) in 25% (v/v) HCl and *N*-(1-napthy) ethylenediamine dihydrochloride solution (NEED): 0.02% (w/v) in distilled water.

- **Antimicrobial activity**

To determine the antibacterial activity of silver nanoparticles, against bacterial strains such as *E.coli*, *Staphylococcus sps*, *Pseudomonas sps*, *and Bacillus sps*. The bacterial cultures were grown overnight in nutrient broth on a rotary shaker (200 rpm) at 37^0C and then they were seeded into nutrient agar plates. Silver nanoparticles with concentration at 100µl were loaded into the wells. The plates were incubated at 37^0C for 24hours, after incubation, zone of inhibition around the wells were measured.

RESULTS AND DISCUSSION

Biosynthesis of Silver nanoparticles by using Actinomycetes isolate

The *Actinomycetes* culture cell filtrate which containing silver ion was incubated in orbital shaker rotating at 200 rpm in dark condition at 26 °C for 72 hours. The *Actinomycetes* incubated with deionized water (positive control) retained its original colour, the silver nitrate treated fungus turned dark brown after 72 h due to the deposition of silver nanoparticles shown in Figure 1 (a, b).

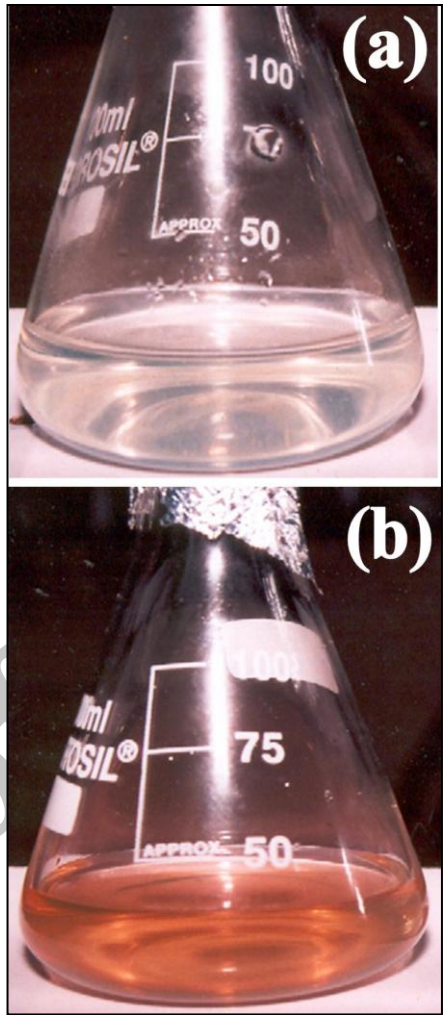

Fig. 1. (a) before additionofAgNO3 (Control) (b)AfteradditionofAgNO372h (Test).

The color change of the fungal filtrate from colorless (negative control) to the dark brown color (Test) on addition of $AgNO_3$ was gives the idea of the formation of the silver nanoparticles.. The generation of dark brown color is due to the surface Plasmon resonance (SPR) exhibited by the nanoparticles The UV–Vis spectrum in Figure 2 Showed an SPR peak of silver nanoparticles at 432 nm. It is well known that the size and shape of the silver nanoparticles reflects the absorbance peak [22- 23]. The SPR peak shifts to longer wavelengths with increase in particle size [24]. The absorption spectrum obtained showed a strong surface Plasmon resonance band maximum at 432nm (Figure 2), a characteristic peak of silver nanoparticles [8].

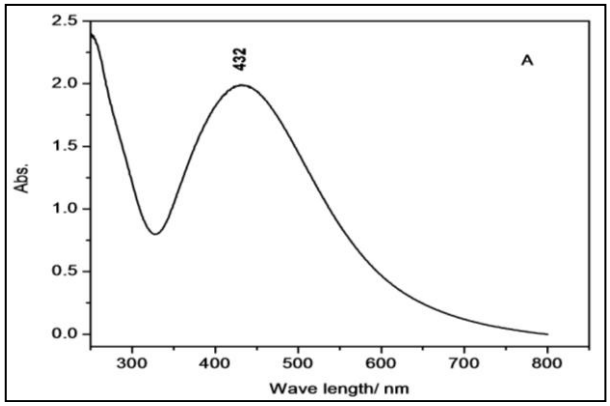

Fig. 2. UV Spectrophotometry of silver nanoparticles.

Characterization of silver nanoparticles by UV-Vis, FTIR and TEM Analysis

The FT-IR spectroscopic study was confirmed that the carbonyl group from amino acid residues and peptides of proteins has the stronger ability to bind to metal (Figure 3). So that, the proteins could most possibly form a coat covering on the metal nanoparticles (Capping of silver nanoparticles) for prevent agglomeration of the particles and stabilizing them in the medium. This evidence suggests that the biological molecules could possibly perform the function for the formation and stabilization of the silver nanoparticles in aqueous medium. The carbonyl groups of to nanoparticles either through free amine or cysteine groups in proteins [25] The proteins present over the silver nanoparticle surface acts as capping agent amino acid residues and peptides have strong ability to bind to silver ion[26] TEM studies Transmission electron microscope image of silver nanoparticles derived from *Actinomycete* was shown in Figure 4. The morphology of the nanoparticles was spherical in nature. The obtained nanoparticles are in the range of sizes approximately 5–50nm and few particles are agglomerated.

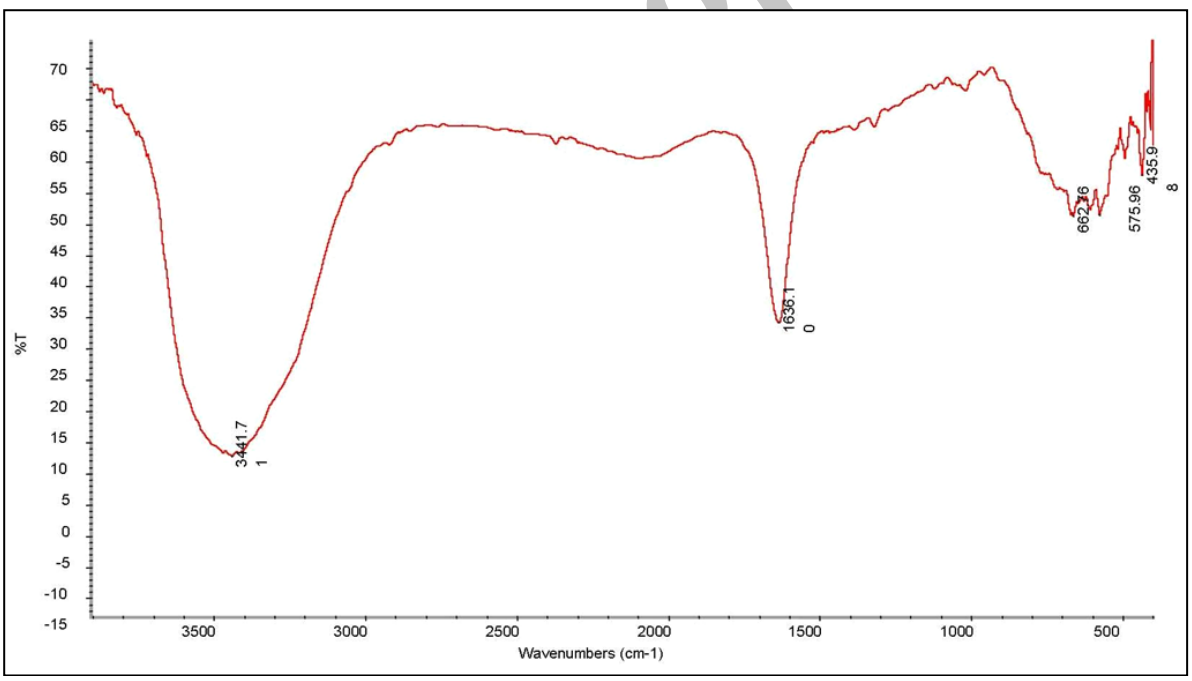

Fig. 3. FTIR analysis of silver nanoparticles

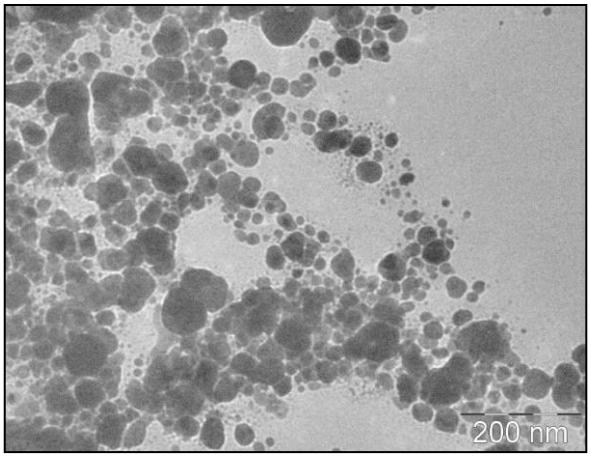

Fig. 4. TEM image of formed silver nanoparticles

Nitrate reductase assay

The Nitrate reductase assay quantifies the amount of enzyme (Nitrate reductase) present in terms of the nitrite generated in the assay. Formation of pink color is positive report for nitrate reductase enzyme in *Actinomycetes*. In this study the amount of nitrate reductase present in the *Actinomycetes* is 120nmol/hr/ml. Previous studies[14-16, 19] have indicated that NADH- and NADH-dependent enzymes are important factors in the biosynthesis of metal nanoparticles. The reduction seems to be initiated by electron transfer from the NADH by NADH-dependent reductase as electron carrier. Similarly Duran *et al*. [27] reported two possible mechanisms for the formation of silver nanoparticles by *Fusarium oxysporum* one is through nitrate reductase and the other by shuttle quinine process. The NADH-dependant nitrate reductase is the main enzyme responsible for the reduction of silver ions to silver in *Fusarium oxysporum* [12] and *Bacillus licheniformis* [28]. Along with extracellular enzymes, several naphthoquinones [29] and anthraquinones [23] with excellent redox properties were reported in *F.oxysporum* that could be act as electron shuttle in metal reductions. It appears that the reductase together with electron shuttling compounds and other peptides/proteins may be responsible for the reduction of Ag^+ ions and the subsequent formation of silver nanoparticles. The synthesized silver nanoparticle exhibited an excellent antibacterial activity against bacterial strains both gram positive and gram negative. The result suggests that silver nanoparticles undergo a interaction with bacterial cell and displayed the strong action against *E.coli* , *Staphylococcus sps*, *Pseudomonas sps*, and *Bacillus* sps. Similarly, Jaydev and Narasimha 2010 [30] and Murali sastry et al., 2003 [31] demonstrated that reactive Antimicrobial activity of metals and metal oxides.

It has been known for a long time that silver ions and silver compounds are highly toxic to most bacterial strains. Recently it was shown that the highly concentrated and non hazardous nanosized silver nanoparticles can easily be prepared in a cost effective manner and tested as a new type of bactericidal and fungicidal nanomaterial agents. In this study, formation of clear zone (restricted bacterial growth) around the cavity is an indication of antibacterial property of silver nanoparticles (Table 1).

Table 1. Inhibitory activity of silver nanoparticles on multidrug resistance bacterial strains

S.No.	Organisms	Zone of inhibition(cm) 100µl
1	*Escherichia. Coli*	1.1
2	*Staphylococcus. Sps*	1.3
3	*Pseudomonas sps*	0.9
4	*Bacillus sps*	1.2
5	Streptomycin (Control)	1.2

*All values represented in the table are average of conducted experiment

CONCLUSIONS

Silver nanoparticles were synthesized from *Actinomycetes* isolated from mangrove soil and these silver nanoparticles were characterized with sophisticated instruments, UV-Vis, FT-IR, TEM and their size and shapes were confirmed. The synthesized silver nanoparticles exhibited an excellent anti bacterial property on multidrug resistance gram positive and gram negative pathogenic bacterial strains.

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Cite this article as: G. Narasimha *et al.*: Extracellular synthesis, characterization and antibacterial activity of silver nanoparticles by Actinomycetes isolative.
Int. J.Nano Dimens. 4(1): 77-83, Summer 2013