Advances in Natural Sciences:
Nanoscience and Nanotechnology

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IOP PUBLISHING
ADVANCES IN NATURAL SCIENCES: NANOSCIENCE AND NANOTECHNOLOGY
Adv. Nat. Sci.: Nanosci. Nanotechnol. **4** (2013) 035005 (5pp)
doi:10.1088/2043-6262/4/3/035005

Trichoderma koningii assisted biogenic synthesis of silver nanoparticles and evaluation of their antibacterial activity

R M Tripathi[1,2,3], Rohit Kumar Gupta[3], Archana Shrivastav[1], M P Singh[4], B R Shrivastav[5,6] and Priti Singh[7]

[1] Department of Microbiology, College of Life Sciences, Gwalior 474 009, M P, India
[2] School of Science in Biotechnology, Jiwaji University, Gwalior 474 011, M P, India
[3] Amity Institute of Nanotechnology, Amity University, Sector 125, Noida 201303, India
[4] Advance Instrumentation Research Facility, Jawaharlal Nehru University, New Delhi 110067, India
[5] Department of Surgical Oncology, Cancer Hospital and Research Institute, Gwalior 474 009, M P, India
[6] Department of Surgery, G. R. Medical College, Palace Road, Gwalior 474009, M P, India
[7] Department of Physics, Manav Rachna College of Engineering, Faridabad, Haryana, India

E-mail: archana_61@yahoo.co.in

Received 22 February 2013
Accepted for publication 10 May 2013
Published 4 June 2013
Online at stacks.iop.org/ANSN/4/035005

Abstract
The present study demonstrates the biosynthesis of silver nanoparticles using *Trichoderma koningii* and evaluation of their antibacterial activity. *Trichoderma koningii* secretes proteins and enzymes that act as reducing and capping agent. The biosynthesized silver nanoparticles (AgNPs) were characterized by UV–Vis spectroscopy, dynamic light scattering (DLS), transmission electron microscopy (TEM) and x-ray diffraction (XRD). UV–Vis spectra showed absorbance peak at 413 nm corresponding to the surface plasmon resonance of silver nanoparticles. DLS was used to find out the size distribution profile. The size and morphology of the AgNPs was determined by TEM, which shows the formation of spherical nanoparticles in the size range of 8–24 nm. X-ray diffraction showed intense peaks corresponding to the crystalline silver. The antibacterial activity of biosynthesized AgNPs was evaluated by growth curve and inhibition zone and it was found that the AgNPs show potential effective antibacterial activity.

Keywords: silver nanoparticles, biosynthesis, antibacterial activity, growth curve, inhibition zone

Classification numbers: 2.05, 4.02

1. Introduction

Manipulation of matter at atomic and molecular scale to produce materials for a desired application is the main aspect of the nanotechnology [1, 2]. The unique properties of nanomaterials due to their high surface-to-volume ratio make them a suitable tool for exploring medical sciences fields such as imaging [3], sensing [4], gene-delivery [5] and targeted drug delivery [6]. A variety of physical [7] and chemical [8] methods are involved in the synthesis of metal nanoparticles. These methods for metal nanoparticles fabrication usually involve toxic chemicals which creates a serious environmental problem [9]. Biological synthesis of nanoparticles has emerged as a viable alternative for physical and chemical methods. Biological synthesis of metal nanoparticles involving the use of plants [1, 2] and micro-organisms [10] is easy, cost-effective and eco-friendly and moreover the synthesized nanoparticles are biocompatible.

Biosynthesis of nanoparticles using fungus is advantageous as compared to plants because fungus

produces more protein which results in high production of nanoparticles [11] and provides longer stability [12]. Extracellular synthesis of silver nanoparticles has been reported by *Fusarium oxysporum* [13], *Aspergillus fumigatus* [14], *Neurospora crassa* [15], etc. Silver nanoparticles (AgNPs) have a wide range of applications in the field of biolabelling [16], antimicrobial filters [17] and bactericidal activity against gram positive as well as gram negative bacteria [18, 19]. The silver nanoparticles can be used as a replacing candidate against the antibacterial agents such as antibiotics which are sometimes toxic and cause irritation [20].

In the present investigation we report the biosynthesis of AgNPs using fungal biomass of *Trichoderma koningii* and evaluation of their antibacterial activity. *Trichoderma koningii* is a non-pathogenic and agriculturally important fungus which antagonises plant pathogens [21]. The antibacterial activity of the biosynthesized silver nanoparticles was analyzed against *Salmonella typhimurium* by growth curve and inhibition zone.

2. Experimental

2.1. Materials and methods

The strains of *Trichoderma koningii* and *Salmonella typhimurium* were obtained from Department of Microbiology, College of Life Sciences, Gwalior, India. Silver nitrate ($AgNO_3$) was purchased from Qualigens Fine Chemicals, Mumbai, India. Malt extract, yeast extract, peptone and glucose were purchased from Hi-media, Mumbai, India.

2.2. Fungal culture

Trichoderma koningii was maintained on PDA slants at $28 \pm 1\,^{\circ}C$. The fungal biomass was obtained by inoculating *Trichoderma koningii* in 100 ml of MYPG medium (malt extract 0.3%, yeast extract 0.3%, peptone 0.3% and glucose 1%) and the pH was maintained at 5.8 ± 1. The fungal culture was then incubated in dark condition at $29\,^{\circ}C \pm 1\,^{\circ}C$ for 120 h under continuous shaking at 200 rpm. After 120 h of incubation the biomass was extracted from the media by centrifugation at 5000 rpm for 15 min followed by extensive washing with distilled water to remove any media components. The washed biomass was made to interact with aqueous solution of silver nitrate.

2.3. Preparation of silver nanoparticles

The silver nitrate (1 mM) solution was prepared in 50 ml deionised water. Fungal biomass (5 g) was brought in contact with the silver nitrate solution in a 200 ml Erlenmeyer flask. The solution was then kept in dark condition at $29 \pm 1\,^{\circ}C$ under continuous shaking at 200 rpm for 72 h. After 72 h of reaction time the colour change was observed.

2.4. Characterization of silver nanoparticles

The formation of AgNPs by the bioreduction of Ag^{+} to Ag^{0} using *Trichoderma koningii* was easily monitored using UV–Vis spectroscopy (UV-1601 pc Shimadzu). The scanning was performed in the range of 200–700 nm. The hydrodynamic size of AgNPs was analyzed by DLS (Zetasizer, Malvern). The morphology and size were determined by TEM (Philips CM-10). A sample for TEM analysis was prepared by drop-coating thin film of AgNPs solution onto the carbon-coated copper grid. The presence of crystallite silver was confirmed through x-ray diffraction. The sample was prepared by drop-coated thin film of biosynthesized AgNPs onto the glass substrate.

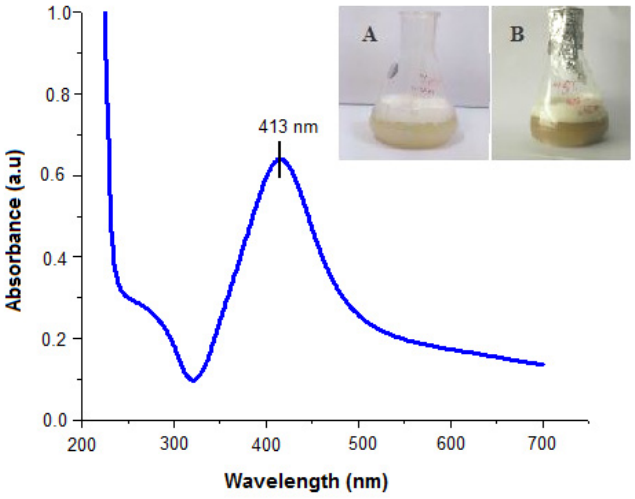

Figure 1. UV–Vis spectra of biosynthesized AgNPs. The inset shows Erlenmeyer flask containing (A) fungal biomass and (B) after reaction between fungal biomass and silver nitrate.

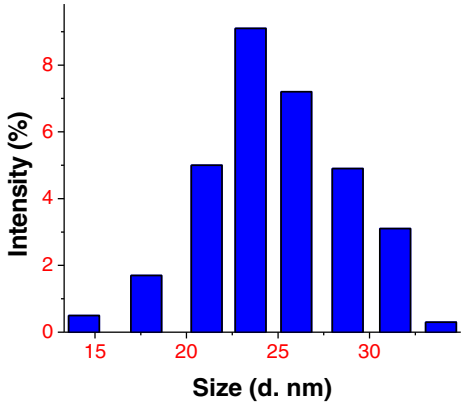

Figure 2. DLS showing size distribution profile of biosynthesized AgNPs.

2.5. Growth inhibition study

2.5.1. Bacterial growth curve. To study the bacterial growth in broth media, fresh colonies on agar media were inoculated in 10 ml of broth (Luria Bertani). This media was supplemented with biosynthesized AgNPs ranges from 20 to 45 $\mu g\,ml^{-1}$ and the bacterial culture was incubated at 37 °C with continuous shaking at 150 rpm. The growth of *Salmonella typhimurium* in broth media was indexed by measuring the optical density (OD) at $\lambda = 600$ nm at regular intervals using UV–Vis spectroscopy. The control culture was treated in a similar fashion but without any exposure to the silver nanoparticles. The growth curve was plotted between optical density and time.

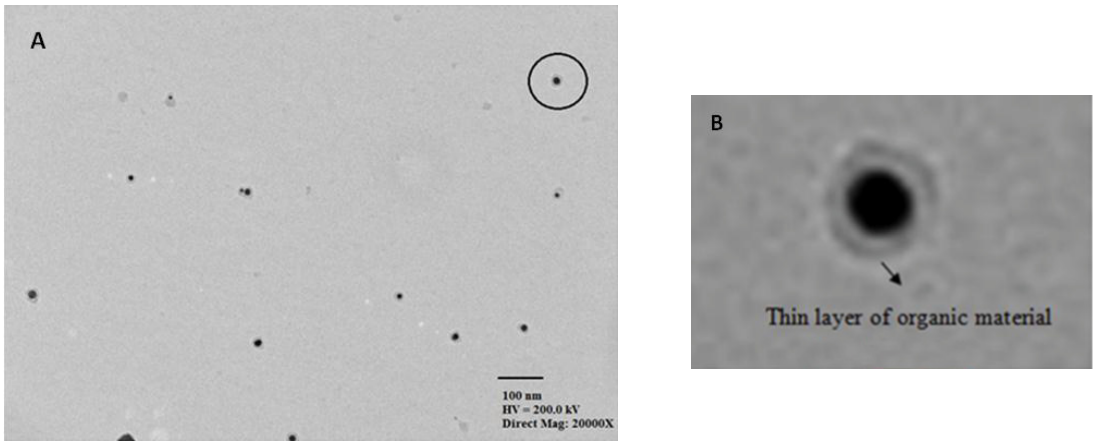

Figure 3. (a) TEM micrograph of biosynthesized AgNPs, (b) AgNP covered by organic material secreted by *Trichoderma koningii*.

2.5.2. Well diffusion method. Well diffusion method was adopted to assay the antibacterial effect of biosynthesized AgNPs against *Salmonella typhimurium*. Four petri-dishes were prepared with Luria Bertani (LB) agar media. The well was created on each petri plate having a diameter of 8 mm and *Salmonella typhimurium* was spread on the LB media. AgNPs solutions were loaded with 2 μg, 5 μg and 7 μg concentration in the wells of three different petri-plates. The control well was also treated in a similar fashion but without any exposure of AgNPs. The plates were incubated at 37 °C for 24 h and the inhibition zone was measured.

3. Results and discussion

3.1. UV–Vis spectroscopy

The reduction of Ag^+ into Ag^0 during exposure to fungal biomass of *Trichoderma koningii* is followed by a gradual increase in colour development from light yellow to yellowish brown, as a result of the surface plasmon resonance phenomenon. It is known that AgNPs show a yellowish brown colour in aqueous solution that arises from the excitation of surface plasmon vibrations in the metal nanoparticles which is a collective excitation of the electrons in the conduction band near the nanoparticles' surface [22, 23]. After 72 h of incubation of the aliquot, the sample was analysed by UV–Vis spectroscopy which shows that the surface plasmon resonance occurred at 413 nm (figure 1).

3.2. Dynamic light scattering

Dynamic light scattering is a technique which determines polydispersity, hydrodynamic sizes and aggregation of particles in suspension. Dynamic light scattering gives the size distribution profile of the biosynthesized AgNPs which comes in the range of 14–34 nm (figure 2). Polydispersity index of the biosynthesized silver nanoparticles was found to be 0.681 which indicates that the nanoparticles are polydispersed in nature.

3.3. Transmission electron microscopy

The nanoparticles were characterized by transmission electron microscopy (TEM) to determine their size and morphology from drop-coated films of the silver nanoparticles synthesized by fungal biomass. TEM micrograph reveals that the nanoparticles are formed in the size range of 8–24 nm with spherical morphology. TEM micrograph indicates the particles are relatively uniform in nature, and also shows that particles are well separated from each other having no agglomeration. TEM was performed at accelerating voltage of 200.0 kV with 20 000× magnification (figure 3(a)). TEM micrograph depicted that the silver nanoparticles are surrounded by a thin layer of other matter. We supposed that this matter is an organic substance which is released by the fungal biomass of *Trichoderma koningii* (figure 3(b)).

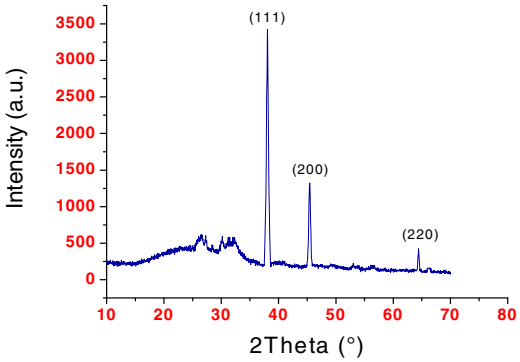

Figure 4. XRD pattern of biosynthesized AgNPs.

3.4. X-ray diffraction

X-ray diffraction (XRD) is commonly used to determine the crystal structure of the nanoparticles. Drop coated film of biogenic AgNPs was prepared for the XRD analysis. Intense peaks occurring at $2\theta = 38.11°$, 44.23° and 64.43 ° corresponds to (111), (200) and (220) set of lattice planes, respectively (figure 4). These Bragg's reflections are corresponding to the planes which are in good agreement with the reference to the face centred cubic structure of the crystalline silver (JCPDS File No. 04–0783). The broadening of the peaks clearly indicates that the particles formed are in nano regime.

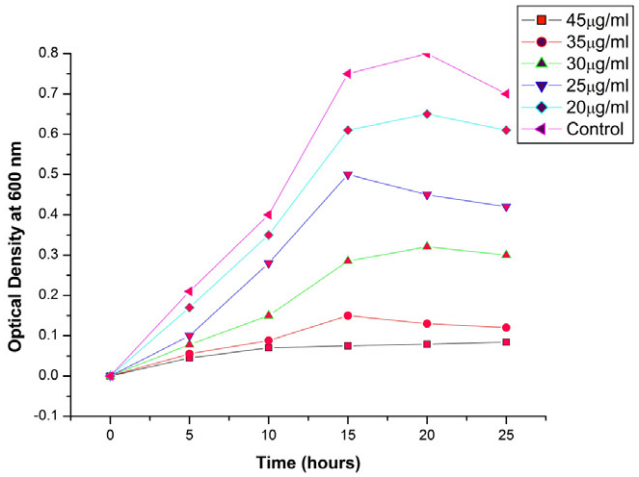

Figure 5. Effect of various concentrations of biosynthesized AgNPs on *Salmonella typhimurium* growth rate.

3.5. Analysis of antibacterial activity

3.5.1. Growth curve. Antibacterial activity of biosynthesized AgNPs was evaluated against *Salmonella typhimurium* by growth rate analysis. Optical densities were measured and plotted as a function of time for 25 h at regular intervals with various concentrations of AgNPs ranging from 20 to 45 μg ml^{-1}. It was observed that optical density of bacterial growth decreases with increasing the concentration of AgNPs (figure 5). It was observed that in the absence of AgNPs, there is an increase of the optical density indicating the increase of bacterial growth, but as the AgNPs concentration increases optical density decreases showing reduction of bacterial growth rate. The optical absorption was insignificant at 45 μg ml^{-1} concentration of AgNPs. This means that at this concentration the bacterial growth does not take place. The minimum inhibitory concentration (MIC) of AgNPs was 25 μg ml^{-1}.

3.5.2. Well diffusion method. After 24 h incubation, no bacterial growth was observed in a particular area around the well, called inhibition zone. The antibacterial activity was evaluated by measuring the diameter of zone. In our experiment it was found that the diameter of zone increases with increasing concentration of AgNPs. The three concentrations of AgNPs were used in this experiment i.e. 2 μg, 5 μg and 7 μg. The inhibition zone was not observed in case of control well (without any exposure of AgNPs). The diameter of zones was found to be 3 mm, 9 mm and 13 mm with 2 μg, 5 μg and 7 μg concentrations of AgNPs, respectively. According to our experimental results, the maximum inhibition zone was found to be 13 mm at 7 μg concentration of AgNPs which indicates that AgNPs show effective antibacterial activity (figure 6).

4. Conclusion

We have developed a biosynthesis method for AgNPs using *Trichoderma koningii* and evaluated their antibacterial activity. The method is cost-effective and eco-friendly because no toxic chemicals were employed. The fungal biomass releases enzymes and proteins which help in production of silver nanoparticles in the size range of 8–24 nm. The biosynthesized silver nanoparticles were characterized by UV–Vis spectroscopy, dynamic light scattering, transmission electron microscopy and x-ray diffraction. The antibacterial activity of the biosynthesized AgNPs was analyzed against *Salmonella typhimurium* and it was found that 45 μg ml^{-1} concentration of AgNPs inhibits bacterial growth completely. The high antibacterial activity of AgNPs was due to its high surface-to-volume ratio.

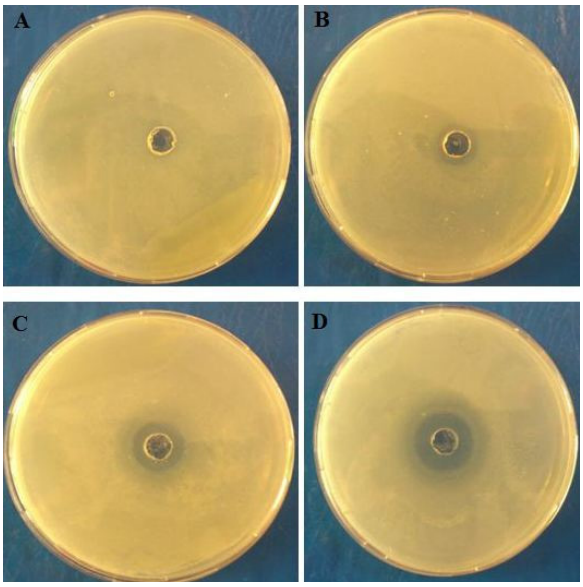

Figure 6. Zone of inhibition against *Salmonella typhimurium* loaded with (A) 0 μg, (B) 2 μg, (C) 5 μg and (D) 7 μg biosynthesized AgNPs.

Acknowledgments

We thank Dr R P Singh and Dr Ashok K Chauhan of Amity Institute of Nanotechnology, Amity University (Noida, India) for their encouragement and providing excellent facilities for the above work. We are also grateful to Professor A M Jana and Deepali Shukla, College of Life Sciences, Gwalior (M.P) for giving their precious advice continuously and for providing the AIRF facilities.

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