International Journal of Advanced Research in Biological Sciences
www.ijarbs.com

Research Article

Synthesis and Characterization of Silver Nanoparticles from *Fusarium oxysporum*

K. Vanmathi Selvi [1]*and T. Sivakumar[2]

[1]Department of Microbiology, Sri Akilandeswari Women's College, Vandavasi, T.V.Malai, Tamil Nadu, India.
[1]Research and Development Centre, Bharathiar University, Coimbatore - 641 046, Tamil Nadu, India
[2]Department of Microbiology, Kanchi shri Krishna College of Arts & Science, Kilambi, Kancheepuram, Tamilnadu, India- 631 551

*Corresponding author e-mail: *kvmsel@gmail.com*

Abstract

A total of 21 isolates of *Fusarium* sp. were isolated from soil samples collected from an area of mangrove forest in Pichavaram, Tamil nadu, India. The isolates were isolated using soil dilution, direct isolation and debris isolation techniques. Based on identification using morphological characteristics, *Fusarium oxysporum* was identified. The extracellular production of silver nanoparticles by the fungus *Fusarium oxysporum* was investigated. It was found that exposure of *Fusarium oxysporum* to silver ion leads to the formation of silver nanoparticles. The silver nanoparticles were in the range of 5-50nm in dimension. The nanoparticles were characterized using UV-Visible spectroscopy, scanning Electron Microscopy (SEM), X-ray Diffractometer (XRD), and Fourier Transform Infrared Spectroscopy (FTIR) analysis. The formation of nanoparticles by this method is extremely rapid, undertaken in ambient conditions and synthesized nanoparticles are stable for several months in the absence of light.

Keywords: Mangrove soil; *Fusarium oxysporum;* Silver nanoparticles; Chacterization of nano particles.

Introduction

Mangrove forests are located at the interface between land and sea, a unique and extreme environment. The soils in mangrove communities are muddy or sandy with loose sediment. They contain submerged mangrove roots, trunks and branches. These conditions attract rich communities of fungi and bacteria. Nanoparticles (NP) are usually clusters of atoms in the size range of 1–100 nm. It is understood that the properties of a metal NP are determined by its size, shape, composition, crystallinity, and structure (Sun and Xia, 2002). As an important metal, silver nanoparticles (AgNPs) have a number of applications, from electronics (Gratzel, 2001) and catalysis (Shiraishi and Toshima, 1999) to infection prevention (White *et al*., 2011) and medical diagnosis (Groneberg *et al*., 2006). For example, AgNPs could be used as substrates for Surface Enhanced Raman Scattering (SERS) to probe single Molecules (Tao *et al*., 2003) and also useful catalysts for the oxidation of

methanol to formaldehyde (Waterhouse *et al.*, 2003). AgNPs has been known as excellent antimicrobial and anti-inflammatory agents, and thus were used to improve wound healing (Elliott, 2010).

To date, a number of physical and chemical strategies were employed for the synthesis of AgNPs (Darroudi, et *al*., 2011; Kilin et *al.*, 2008). However, concern has been raised on the toxicity of chemical agents used in AgNPs synthesis. Thus, it is essential to develop a green approach for AgNPs production without using hazardous substances to the human health and environment. Compared with the traditional synthetic methods, biological systems provide a novel idea for the production of nano-materials (Bansal *et al*., 2011). Up to now, several microorganisms from bacteria to fungi have been reported to synthesize inorganic materials either intra- or extracellularly, and thus to be potentially utilized as eco-friendly nanofactories (Shankar *et al*., 2004; Mohanpuria, *et al*., 2008). *Pseudomonas stutzeri* AG259, isolated from silver mines, has been shown to produce silver nanoparticles (Klaus *et al*., 1999), and the bioreduction of Ag was also reported in *Bacillus licheniformis.*

Recently a further advancement in the biological synthesis approach was shown by demonstrating that the shape of Ag nanoparticles could be tuned from nanospheres to nanoprisms by controlling the growth kinetics of a silver resistant bacteria *Morganella psychrotolerans (*Ramanathan *et al*., 2011). Moreover, the same research group also demonstrated that all the members of the genus *Morganella* were capable of synthesizing extracellular Ag nanoparticles, which was correlated to silver resistance machinery operating in these organisms (Parikh, *et al*., 2011) Compared with bacteria, fungi have been known to secrete much higher amounts of bioactive substances, which made fungi more suitable for large-scale production (Narayanan and Sakthivel, 2010).

In addition, the extracellular biosynthesis using fungi could also make downstream processing much easier than bacteria. An interesting example of the biosynthesis using fungi was that the cell-associated biosynthesis of silver using *Fusarium oxysporum* was demonstrated (Ahmad *et al*., 2003) and the particles were overall quasi-spherical with size range between 5 and 15 nm (Ahmad, *et al*., 2003). There also have been several reports on the biosynthesis of AgNPs using fungi, including *Fusarium acuminatum* (Ingle, *et al*., 2008) and *Penicillium fellutanum* (Kathiresan *et al.*, 2009). Despite these impressive results, the origins of fungi having the ability for AgNPs synthesis were still limited, and the detailed mechanism was still not well elucidated. Previous reports have shown that a large number of active substances secreted by fungi played important roles as reducing agents and capping agents in the reaction (Bharde, *et al*.,2006). Therefore, it was of great significance to explore novel fungi strain for synthesizing AgNPs based on the biodiversity. More importantly, it could also facilitate the deeper understanding of molecular mechanism for AgNPs biosynthesis.

Herein, we investigated the biosynthesis of AgNPs using *Fusarium oxysporum* and its underlying mechanism. The properties of obtained AgNPs were characterized by ultraviolet-visible spectroscopy, transmission electron microscopy (TEM) and X-ray diffraction (XRD) techniques. This work provided a potential for the production of AgNPs without the involvement of toxic chemicals and radiation.

Materials and Methods

Study area and Sample Collection

Soil sample were collected from an area of mangrove forest in Pichavaram, Tamil Nadu, India. The soil sample were taken from a depth of 5-10 cm and then kept in plastic bags until drying was performed immediately after sampling in the laboratory.

Sample Processing

The soil samples were air dried at room temperature (27±1°C) for seven days and then ground using a mortar and pestle. Ground soil samples were sieved with a 0.5mm sieve to remove larger particle such

as stone and plant debris in order to obtain a consistent soil particle size for isolation using the soil dilution technique. Silver soils and debris were then store separately in paper bags and kept at 4°C.

Isolation and identification of *Fusarium oxysporum*

The method were used for isolation of *Fusarium* isolates from the mangrove soil samples namely, soil dilution, debris isolation and direct isolation techniques. The Morphological characteristics are identified by Microscopic methods isolates which appeared morphologically different were selected and purified and maintained on PDA slant stored at 4°C until further use.

Extracellular synthesis of silver nanoparticles

Production of Biomass

To prepare the biomass for synthesis, the *Fusarium oxysporum* obtained was grown aerobically in liquid broth containing malt extract powder, glucose, yeast extract and peptone. The culture flasks were incubated on room temperature at 27°C. The biomass was harvested after 120 hours of growth by sieving through a plastic sieve followed by extensive washing with sterile double distilled water to remove any medium components from the biomass.

Synthesis of AgNPs

Typically 10g of biomass (wet weight) were brought in to contact with 100ml sterile double distilled water for 48 hours at 27°C in a Erlenmeyer flask and agitated 120rpm. After incubation the cell filtrate was filtered by whatman filter paper No.1. The filtrate was treated with 1mm $AgNO_3$ solution in an Erlenmeyer flask and incubated at room temperature in dark. Control containing cell free filtrate without silver nitrate solution was run simultaneously as standard with the experimental flask. AgNPs were concentrated by centrifugation of the reaction mixture at 10,000rpm Change in color of the cell free filtrate incubated with silver nitrate solution was visually observed over a period of time. The bio-solution of precursor silver ions was monitored by sampling of Oliquots (1ml) at different time intervals. Absorption measurements were carried out on UV-Visible spectrophotometer at a resolution of 1nm.UV-Visible analysis of several weeks old samples was also carried out to check the stability of synthesized AgNPs.

Characterization of silver nitrate Nanoparticles

UV-Visible Spectroscopy Analysis

This coater (JEOL, Japan, Model No. JFC-1600) which consists of a main unit and a pump is intended mainly to prepare specimen for SEM observation. It coats biological and other nonconductive specimens with metals, efficiently in a short time. The cathode contains a permanent magnet to create an efficient glow discharge for sputtering. It is possible to set the chamber pressure in addition to the sputtering current. This enables the uniformity of the coating, so that a shadow-free coating can be obtained. Operation is easy, and a fine coating of platinum can be obtained in a short time. By using an optional film thickness monitor and a film thickness controller, it is possible to obtain a more accurate film thickness.

Scanning Electron Microscope

This study was undertaken o know the size and shape of the silver nanoparticles biosynthesized using *Fusarium oxysporum*. The images of nanoparticles were obtained in the scanning electron microscope.

X Ray Diffraction studies

The air dried nanoparticles were coated onto XRD grid and analyzed for the formation of silver nanoparticle by Philips X-Ray Diffractometer with Philips PW 1830 X-Ray Generator operated at a voltage of 40kv and current of 30mA with cu kal radiation. The diffracted intensities were recorded from 10° to 80° of 20 angles.

FTIR Spectroscopy Analysis

For Fourier Transform Infra Red (FTIR) spectroscopy measurement, the bio-transformed product present in cell-free filtrate were freeze-dried and diluted with potassium bromide in the ratio of 1:100 FTIR spectrum of samples was recorded in Nicolet Impact 400FT-IR spectrophometer instrument with a diffuse reflectance mode (DRS-8000) attachment. All measurement were carried out in the range of 400-4000´cm at a resolution of 4cm´.

Transmission Electron Microscope

For TEM measurements, a drop of synthesized AgNPs was placed on the carbon coated copper grids and kept overnight under vacuum desiccation before loading them on to a specimen holder. TEM micrographs of the sample were taken using the JEOL JSM 100cx TEM instrument operated at an accelerating voltage of 200kv.

Results and Discussion

Fusarium oxysporum was isolated from the mangrove soil samples (Figures.1, 2). These potent fungi were tentatively identified based on the morphological and Microscopical observations. These cultures were maintained in PDA (potato dextrose agar) medium and transferred to MGYP (Maltase glucose yeast peptone) medium for the synthesis of silver nanoparticles (Fig.3). The colour of the fungus culture changed from its natural colour to yellowish brown.

Surface Plasmon Resonance of reduced silver nanoparticles

Aqueous Silver nitrate ions were reduce during exposure to the *Fusarium oxysporum* cell filtrate. The colour of the reaction mixture changed from pale yellow to yellowish brown as shown in figure, which indicates the formation of silver nanoparticles (Fig.3).

It is well known that silver nanoparticles exhibit yellowish brown colour in water due to excitation of surface Plasmon vibration in metal nanoparticles. Control (without silver nitrate) shows no colour change with aqueous silver nitrate solution when incubated at same condition. Control showed pale yellow colour solution with culture filtrate and Silver nanoparticles showed Dark brown colour solution after 24 hrs of incubation. Formation of silver nanoparticles by reduction with $AgNO_3$ (Silver nitrate) by *Fusarium oxysporum* cell filtrate samples were characterized by UV-Visible spectroscopy and this technique has proved to be very useful for the analysis of nanoparticles (Fig.4). Stability of synthesized was monitored regularly for about 3 months. It was observed that the nanoparticles solution was extremely stable at room temperature, with no of flocculation of particles as determined by UV-Visible spectroscopy measurements. This indicated that the nanoparticles were well dispersed in the solution without aggregation.

Transmission Electron Microscopy studies

TEM provide further insight into the morphology and particle size distribution profile of the AgNPs and revealed pattern similar to the biosynthesized AgNPs characterized using TEM. The data obtained from transmission electron- micrograph showed distinct shape and size of nanoparticles. The particle were spherical in shape in the range of 5~50nm and uniformly distributed without significant agglomeration (Fig. 5).

Scanning Electron Microscopy studies

The SEM micrograph shows silver nanoparticles aggregates. In this micrograph observed spherical nanoparticles in the size range 20-50nm. The nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a capping agent (Fig.6).

XRD analysis

XRD analysis showed three distinct differaction peaks at 38.28°, 44.38°,64.54° and 77.64° and can be indexed 20 values of (111), (200), (220), (311) crystalline planes of cubic Ag. The average grain nsize of the silver nanoparticles formed in the bioreduction process is determined using scherr's

Figure. 1 *Fusarium oxysporum* on PDA plate

Figure. 2 Microscopic observation of *Fusarium oxysporum*

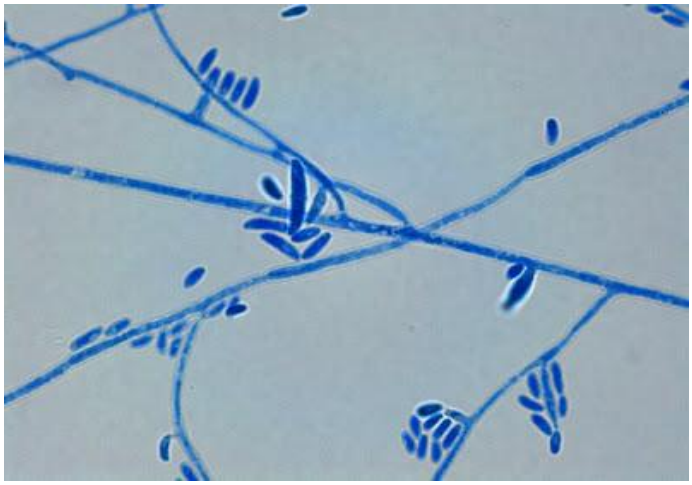


Figure.3 Synthesis of silver nanoparticles

Figure.4 UV-Visible absorption spectra obtained for silver nanoparticles synthesised by *Fusarium oxysporm*

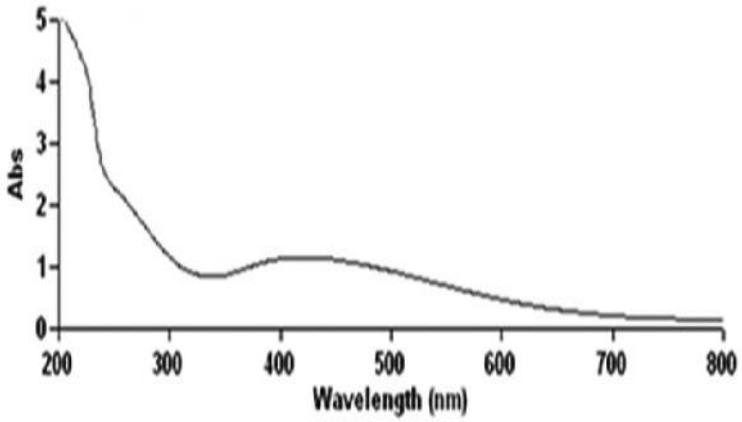


Figure 5. Transmission electron micrograph of the silver nanoparticles synthesized by *Fusarium oxysporum*

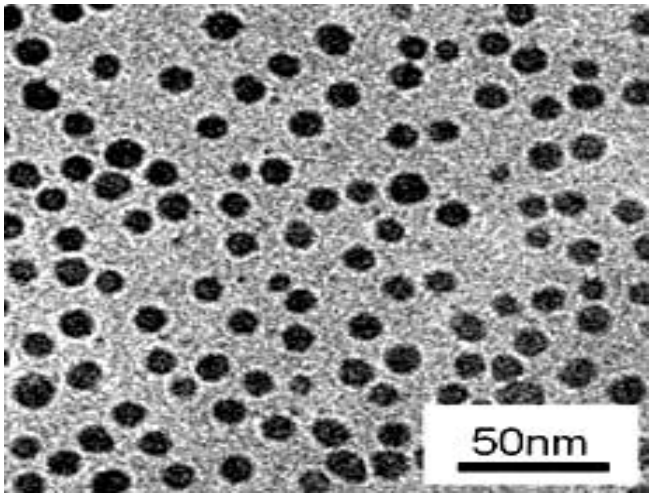


Figure 6: Scanning electron micrograph of silver nanoparticles

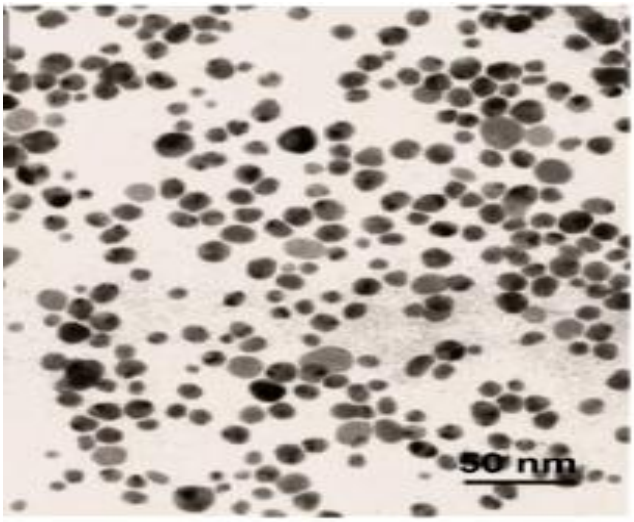


Figure. 7 Shows the XRD pattern of the silver nanoparticles formed in our experiment.

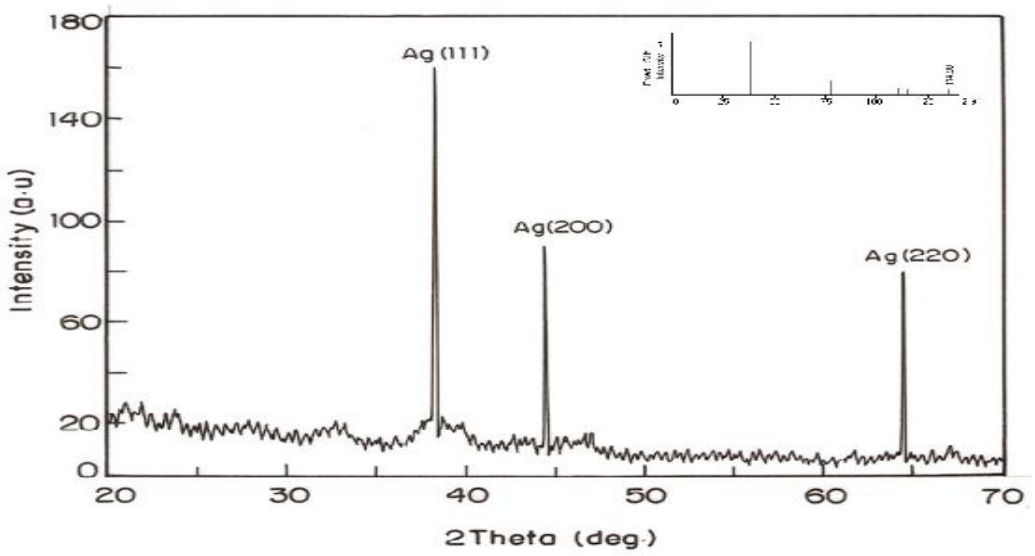
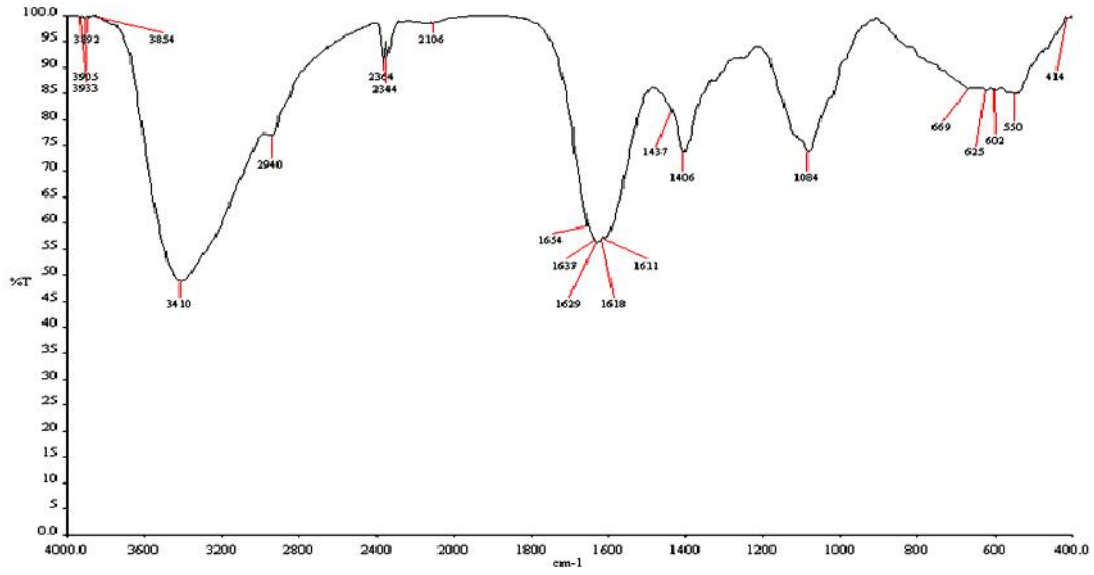


Figure 8. FTIR spectra recorded from powder of silver nanoparticles



formula d=(0.9λ* 180)/βCosθπ and is estimated to be 5.2nm (Fig.7).

FTIR analysis

FTIR measurements of the freeze-dried samples were carried out to identify the possible interactions between silver and bioactive molecules, which may be responsible for synthesis and stabilization (capping material) of silver nanoparticles. The amide linkages between amino acid residues in proteins give rise to well known signatures in the infrared region of the electromagnetic spectrum. FTIR spectrum reveals two bands at 1647 and 1543 cm_1 that corresponds to the bending vibrations of the amide I and amide II bands of the proteins respectively; while their corresponding stretching vibrations were seen at 3302 and 2926 cm_1 respectively (Fig. 8). The presence of the signature peaks of amino acids supports the presence of proteins in cell-free filtrate as observed in UV-Vis. spectra.

It is well known that protein–nanoparticle interactions can occur either through free amine groups or cysteine residues in proteins and via the electrostatic attraction of negatively charged carboxylate groups in enzymes.30 The two bands observed at 1381 and 1032 cm_1 can be assigned to the C–N stretching vibrations of the aromatic and aliphatic amines, respectively. These observations indicate the presence and binding of proteins with silver nanoparticles which can lead to their possible stabilization. FTIR results revealed that secondary structure of proteins have not been affected as a consequence of reaction with silver ions or binding with silver nanoparticles. It is important to understand though, that it is not just the size and shape of proteins, but the conformation of protein molecules that plays an important role.

References

Ahmad, A., P. Mukherjee, S. Senapati, D. Mandal, M.I. Khan, R. Kumar and Sastry. M. 2003. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. Colloid Surface B. 28: 313–318.

Bansal, V., R. Ramanathan and Bhargava, S.K. 2011. Fungus-mediated biological approaches towards "green" synthesis of oxide nanomaterials. Aust. J. Chem. 64: 279–293.

Bharde, A., D. Rautaray, V. Bansal, A. Ahmad, I. Sarkar, S.M. Yusuf, M. Sanyal and Sastry, M. 2006. Extracellular biosynthesis of magnetite using fungi. Int. J. Mol. Sci. 2: 135–141.

Darroudi, M., M.B. Ahmad, A.K. Zak, R. Zamiri and Hakimi, M.2011. Fabrication and characterization of gelatin stabilized silver nanoparticles under UV-light. Int. J. Mol. Sci. 12: 6346–6356.

Elliott, C., 2010. The effects of silver dressings on chronic and burns wound healing. Br. J. Nurs. 19: S32–S36.

Gratzel, M., 2001. Photoelectrochemical cells. Nature. 414: 338–344.

Groneberg, D.A., M. Giersig, T. Welte and Pison, U.2006. Nanoparticle-based diagnosis and therapy. Curr. Drug Targets. 7: 643–648.

Ingle, A., A. Gade, S. Pierrat, C. Sonnichsen and Rai, M. 2008. Mycosynthesis of silver nanoparticles using the fungus *Fusarium acuminatum* and its activity against some human pathogenic bacteria.Curr. Nanosci. 4: 141–144.

Kathiresan, K., S. Manivannan, M.A. Nabeel and Dhivya, B. 2009. Studies on silver nanoparticles synthesized by a marine fungus, *Penicillium fellutanum* isolated from coastal mangrove sediment. Colloid Surface B.71: 133–137.

Kilin, D.S., O.V. Prezhdo and Xia, Y.N. 2008. Shape-controlled synthesis of silver nanoparticles: Ab in vitro study of preferential surface coordination with citric acid. Chem. Phys. Lett. 2008, 458, 113–116.

Klaus, T., R. Joerger, E. Olsson and Granqvist, C.G.1999. Silver-based crystalline nanoparticles, microbially fabricated. Proc. Natl. Acad. Sci. USA. 96: 13611–13614.

Mohanpuria, P., N.K. Rana and Yadav, S.K. 2008. Biosynthesis of nanoparticles: Technological concepts and future applications. J. Nanopart. Res. 10: 507–517.

Narayanan, K.B., and Sakthivel, N. 2010. Biological synthesis of metal nanoparticles by microbes. Adv. Colloid Interface Sci. 156: 1–13.

Parikh, R.Y., R. Ramanathan, P.J. Coloe, S.K. Bhargava, M.S. Patole, Y.S. Shouche and Bansal, V. 2011. Genus-wide physicochemical evidence of extracellular crystalline silver nanoparticles biosynthesis by *Morganella* spp. PLoS One. 6, e21401.

Ramanathan, R., A.P. O'Mullane, R.Y. Parikh, P.M. Smooker, S.K. Bhargava and Bansal, V.2011. Bacterial kinetics-controlled shape-directed biosynthesis of silver nanoplates using Morganella psychrotolerans. Langmuir.27: 714–719.

Shankar, S.S., A. Rai, B. Ankamwar, A. Singh, A. Ahmad and Sastry, M. 2004. Biological synthesis of triangular gold nanoprisms. Nat. Mater. 3: 482–488.

Shiraishi, Y., and N. Toshim. 1999. Colloidal silver catalysts for oxidation of ethylene. J. Mol. Catal. A. 141: 187–192.

Sun, Y.G., and Xia, Y.N. 2002. Shape-controlled synthesis of gold and silver nanoparticles. Science. 298: 2176–2179.

Tao, A., F. Kim, C. Hess, J. Goldberger, R.R. He, Y.G.Sun, Y.N. Xia and Yang, P.D.2003.

Langmuir-blodgett silver nanowire monolayers for molecular sensing using surface-enhanced raman spectroscopy. Nano Lett. 3:1229–1233.

Waterhouse, G.I.N., G.A. Bowmaker and Metson, J.B. 2003. Oxygen chemisorption on an electrolytic silver catalyst: A combined TPD and raman spectroscopic study. Appl. Surf. Sci. 214: 36–51.

White, R.J., V.L. Budarin, J.W.B. Moir and Clark, J.H. 2011. A sweet killer: Mesoporous polysaccharide confined silver nanoparticles for antibacterial applications. Int. J. Mol. Sci. 12: 5782–5796.