Mycopath (2013) 11(1): 1-7 **Research Article**

Silver nanoparticles biosynthesis by *Fusarium moniliforme* and their antimicrobial activity against some food-borne bacteria

[*]T.M. Abdel Ghany[1, 2], Abdel Rhaman M. Shater[1], M.A. Al Abboud[1], M.M. Alawlaqi[1]

[1]Biology Department, Faculty of Science, Jazan University Kingdom of Saudi Arabia
[2]Botany and Microbiology Department, Faculty of Science, AL-Azhar University, Cairo, Egypt
[*]Corresponding author's email: tabdelghany@yahoo.com

Abstract

The biosynthesis of metal nanoparticles is an expanding research area due to the potential applications for emerging ecofriendly science. The present study proved a rapid and extracellular biosynthesis of silver nanoparticles (AgNPs) by a fungus, *Fusarium moniliforme,* isolated from infected onion. Upon addition of the silver ion into the flask containing the mycelial mat, the color of the medium changed to brown, typical of the AgNPs. The AgNPs showed maximum absorbance at 420 nm on ultraviolet-visible spectra. The transmission electron micrograph revealed the formation of AgNPs with an average size of about 50-100 nm. The presence of proteins was identified by Fourier transform-infrared spectroscopy. Combination between AgNPs and ciprofloxacin was evaluated for their antimicrobial activities, and the antibacterial activity of ciprofloxacin was increased in the presence of AgNPs against some food borne bacteria i.e. *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*.

Keywords: Antimicrobial activity, Biosynthesis, Silver Nanoparticles.

Introduction

Development of reliable and eco-friendly process for synthesis of metallic nanoparticles is an important step in the field nanotechnology (Prashant and Raja, 2011). However, nanotechnology is currently fast growing niche in the field of nanoscience (Mandal *et al*., 2006; Kalishwaralal *et al*., 2008). An important area of research in nanotechnology is the biosynthesis of nanoparticles such as nanosilver. The first evidence of synthesizing silver nanoparticles (AgNPs) was established in 1984 using the microorganism *Pseudomonas stutzeri* AG259, a bacterial strain that was originally isolated from silver mine (Haefeli *et al*., 1984; Nair and Pradeep, 2002; Zhang *et al*., 2005). At present, chemical methods are discouraged due to capital cost, toxicity, non eco-friendly and low productivity (Kowshik *et al*., 2003). Biosynthetic methods have been investigated as an alternative to chemical and physical ones. These methods can be divided into two categories depending on the place where nanoparticles were created as many microorganisms can provide inorganic materials either intra- or extra-cellularly (Riddin *et al*., 2006; Singaravelu *et al*., 2007; Ingle *et al*., 2009). The extra cellular production of nanoparticles by several strains of *Fusarium oxysporum* has been described by Duran *et al*. (2005). The extra cellular synthesis of stable AgNPs using the fungus *Aspergillus flavus* has also been reported (Vighneshwaan *et al.,* 2007). Different fungi such as *Verticillium*, *F. oxysporum* and *Colletotrichum* sp. have been reported to synthesize metal nanoparticles (Ahmad, 2003; Mandal *et al*., 2006; Mohammed *et al*., 2009). On the other hand many authors reported that AgNPs were synthesized using bacteria (Minaeian *et al*., 2008; Shirley *et al*., 2010; Arun *et al*., 2013). The filamentous fungi possess some advantages over bacteria in nanoparticles synthesis, as most of the fungi are easy to handle, required simple nutrient, possess high wall-binding capacity, as well as intracellular metal uptake capabilities (Sanghi and Verma, 2009). Sastry *et al*. (2003) have reported that fungi when exposed to gold and silver ions, reduced the metal ion fairly rapidly and formed respective metallic nanoparticles.

The silver and certain other noble metal nanoparticles have several important applications in the field of biolabelling, sensors, drug delivery system (Mann and Ozin, 1996), filters and

possesses antimicrobial activity (Ingle *et al*., 2008). With the prevalence and increase of microorganisms resistant to multiple antibiotics and the continuing emphasis on health care costs, many scientific papers have tried to develop new, effective antimicrobial agents free of resistance along cost-effectiveness. Such problems and needs have led to the resurgence in the use of silver-based antiseptics that may be linked to broad-spectrum activity and far lower propensity to induce microbial resistance than antibiotics (Jones *et al*., 2004). Piksova *et al*. (2009) studies the bactericidal effect of AgNPs in the range of 10-30 nm on Gram negative bacteria and Gram-positive bacteria. Recent reports indicate that fungi (Kim *et al*., 2009; Velmurugan *et al*., 2009), HIV virus (Elechiguerra *et al*., 2005), bacteriophage viral strain (Narasimha *et al*., 2012) are susceptible to AgNPs. Moreover, Silver in the form of nanoparticles that release silver ions more effectively has a high bactericidal activity due to its high surface-area-to-volume ratio (Duran *et al*., 2010; Sheet *et al*., 2013) and the fact that it can be easily deposited on solid materials for the deactivation of microorganisms in water treatment (Nair and Pradeep, 2007). Among different fungi species of Fusarium like *F. oxysporum*, *Fusarium acuminatum* and *F. solani* have been used for nanoparticles synthesis (Duran *et al*., 2005; Ingle *et al*., 2008; Ingle *et al*., 2009). So far, *F. moniliforme* is a well known phytopathogen and causes infection in many economically important plants pre-and post harvest. The exploitation of such phytopathogen is beneficial because it helps to study and detect the infections in plants as well as useful in nanoparticle synthesis. The aim in the present contribution was to synthesize AgNPs using *F. moniliforme* and to investigate the synergistic effect of AgNPs combined with antibiotics against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi.*

Materials and Methods

Isolation and identification of *Fusarium moniliforme*

Isolation of *F. moniliforme* was carried out from infected onion (collected from different markets), maintained on potato dextrose agar (PDA) medium at 28 °C and stored at 4 °C for further study. The isolated fungus was identified on the basis of their morphological characteristics and microscopical examination according to Domsch and Gams (1972), Domsch *et al*. (1980) and John and Brett (2006) (Fig. 1).

Biosynthesis of silver nanoparticles

F. moniliforme was inoculated on potato dextrose broth medium and incubated at 28°C for 10 days. The biomass was harvested after complete incubation by filtering through filter paper followed by repeated washing with distilled water to remove any medium component from the biomass. Ten grams (wet weight) was brought in contact with 100 mL of sterilized double distilled water treated with aqueous 1 mM $AgNO_3$ for 72 h at 28 °C in a 250 mL Erlenmeyer flask and agitated again at 120 rpm. Control (without the silver ions) was also run along with the experimental flask.

UV-visible spectroscopic analysis

The reduction of silver ions was confirmed by qualitative testing of supernatant by UV-visible spectrophotometer. One ml of sample supernatant was withdrawn after 24 hours and absorbance was measured by using UV-visible spectrophotometer between 300-800 nm at the Regional Center for Mycology and Biotechnology (RCMB) at Al-Azhar University Cairo, Egypt**.**

Fourier Transform Infrared Spectroscopy and Transmission Electron Microscopy analysis

The dried powder of AgNPs was subjected to Fourier Transform Infrared Spectroscopy (FTIR) analysis. Two milligrams of the sample was mixed with 200 mg KBr (FTIR grade) and pressed into a pellet. The sample pellet was placed into the sample holder and FT-IR spectra were recorded in the range 450- 4000-500 cm^{-1} in FTIR spectroscopy at a resolution of 4 cm^{-1}. Finally, the AgNPs were characterized by Transmission Electron Microscopy (C Joel Jem-1200 EX II. Acc. Voltage 120 KV. MAG-medium) at RCMB.

Antimicrobial activity

The antimicrobial activity of AgNPs was tested against *B. subtilis*, *S. aureus*, *E. coli* and *S. typhi*. Cavities of 6 mm were made in culture media inoculated with the test organism and were filled with 500 µL of AgNPs. To determine the synergistic effects, cavities of 6 mm were made in culture media and filled with 500 µL of AgNPs and10 µg of ciprofloxacin. After incubation period, the inhibition zone (mm) was recorded. The cavities filled with double sterilized distilled water served as control.

Results and Discussion

Initially, the synthesis of AgNPs by *F. moniliforme* was confirmed by observing the

change in color of the reaction mixture. The appearance of a brown color from colorless cell filtrate suggested the formation of silver nanoparticles, which corroborate the results obtained by Ahmad *et al*. (2003) (Fig. 2). The intensity of the color varies with the incubation period. The generation of dark brown color is due to the surface plasmon resonance exhibited by the nanoparticles (Jaidev and Narasimha, 2010; Prashant and Raja, 2011; Honary *et al*., 2013).

The confirmation of formation and stability of the AgNPs in the colloidal solution was monitored by using UV-Vis spectral analysis. Fungal biomass treated with silver nitrate (1 mM) showed the sharp peak at around 420 nm, which is very specific for AgNPs **(**Fig. 3). This result agreement with many authors (Ahmad *et al.*, 2003; Ingle *et al*., 2008; Ingle *et al*., 2009; Honary *et al*., 2013).

The results of scanning electron microscopy (SEM) showed that the AgNPs have a rod shape, with an average size of about 50-100 nm (Fig. 4). Size of AgNPs was 10-100, 5-40, 200 and 500 nm produced by *Lactobacillus* strains (Nair and Pradeep, 2002), *Cladosporium cladosporioides* (Balaji *et al*., 2009) and *Trichoderma viride* (Fayaz *et al*., 2010). Previously, Gurunathan *et al*. (2009) have reported the size-controlled synthesis of AgNPs by controlling the temperature and pH of the supernatant of *E. coli*.

FTIR measurements were carried out to identify the possible biomolecules responsible for the reduction of the silver ions in nanoparticles formed by *F. moniliforme* (Fig. 5). Representative spectra recorded showed absorption peaks at about 1465.8, 1643.2, 2067.5, 3417.6 and 3830.4 cm^{-1}. The FTIR spectra revealed the presence of different functional groups like C=C at peak 1643.2 and –COO at peak 1465.8 according to Huang *et al*. (2007) and Shankar *et al*. (2003). Basavaraja *et al*. (2008) reported that the peak at 1636.17cm^{-1} was due to the carbonyl stretch vibrations in the amide linkages of proteins. FTIR spectrum supported the presence of proteins in the synthesis of AgNPs.

The AgNPs also exhibited the antibacterial activity against both gram-positive i.e. *B. subtilis* and *S. aureus* and gram-negative viz., *E. coli*. and *S. typhi*. Zone of inhibition measuring 0, 14.0, 15.0 and 17.0 mm was observed for *S. typhi*. *B. subtilis, S. aureus* and *E. coli*, respectively (Table 1). Similar observations were made by Sondi and Sondi and Sondi (2004) and Jaidev and Narasimha (2010). Beveridge and Fyfe (1985) stated that cell walls of Gram positive bacteria were found to bind with large quantities of metals than the Gram negative bacteria. The mechanism of inhibitory action caused by AgNPs against *E.coli* is partially known, where some researchers reported that AgNPs inhibit bacterial growth through binding to the thiol group leading to bacterial inactivation (Yan *et al*., 2012). Moreover, the Gram negative bacteria have a layer of lipopolysaccharides at the exterior that are composed of covalently linked lipids and polysaccharides; they lack strength and rigidity (Guzman *et al*., 2008). In the current result, the synergistic effect against all test bacterial strains was reported as a result of the addition of AgNPs to antibiotic ciprofloxacin. The highest percentage of antibacterial activity of ciprofluxacin was recorded against *S. typhi* followed by *E. coli*, *S. aureus* and *B. subtilis* (Table 1). The synergistic effect of AgNPs with antibiotics was reported recently (Fayaz *et al*., 2010).

Table 1. Antimicrobial activity of silver nanoparticles and ciprofloxacin against Gram-positive and Gram-negative bacteria.

Silver nanoparticles (AgNPs)	**Antibacterial activty (inhibition zone mm)**			
	Gram-positive		Gram-negative	
	B. subtilis	*S. aureus*	*E. coli*	*S. typhi*
Control	0.00	0.00	0.00	0.00
AgNPs	14.0	15.0	17.0	0.0
Ciprofloxacin (a)	25.0	24.0	21.0	20.0
AgNPs + Ciprofloxacin (b)	26.0	27.0	25.0	24.00
Fold increase (%) = (b − a)/a) × 100	4.00	12.50	19.04	20.00

The present study concludes that *F. moniliforme* is capable of producing AgNPs extracellularly. AgNPs formed by *F. moniliforme* showed antibacterial activity and increased antibacterial activity of ciprofloxacin. Future studies can be conducted to explore further applications of the AgNPs.

Acknowledgement

Thank to Dr. Mahmoud Al-Assar, researcher in Regional Center of Mycology and Biotechnology, Al Azhar University, Cairo, Egypt for providing laboratory facilities.

Fig. 1: *F. moniliforme* on infected onion, colony and their macroconidia.

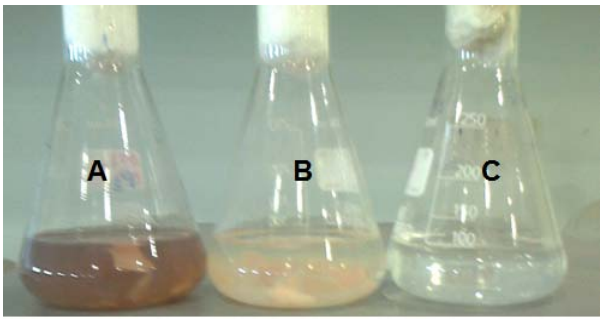

Fig. 2: Conversion of silver nitrate to nano silver (A) by *F. moniliforme* (A, silver nitrate solution inoculated with fungus biomass; B, distilled water inoculated with biomass of fungus; C, silver nitrate solution without fungus biomass).

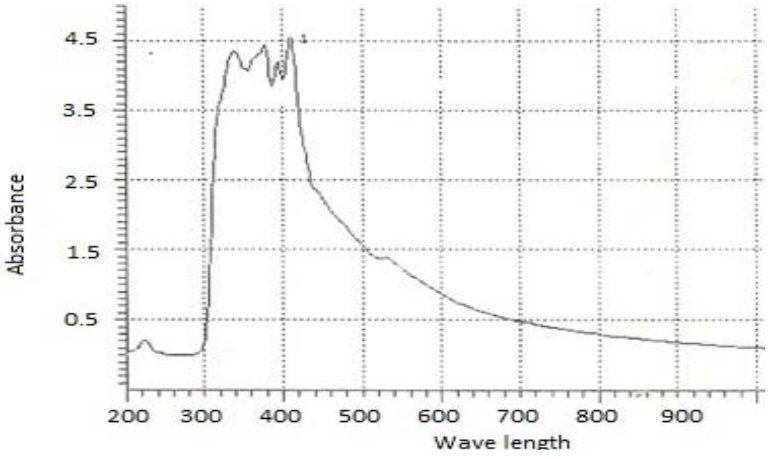

Fig. 3: UV-Vis spectrum of silver nanoparticles produced by *F. moniliforme*.

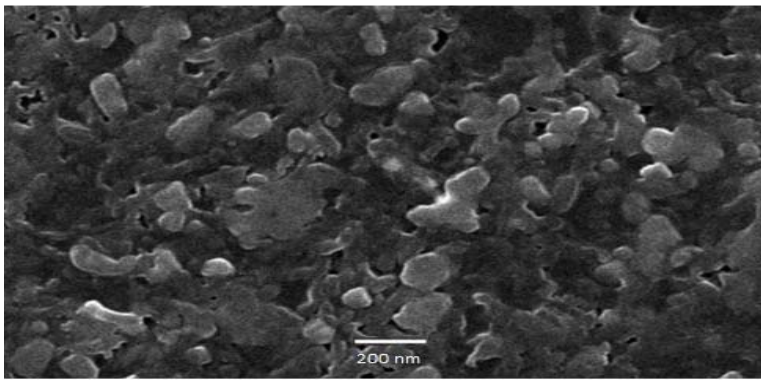

Fig. 4: Characterization of AgNPs with different magnification power using Transmission Electron Microscopy.

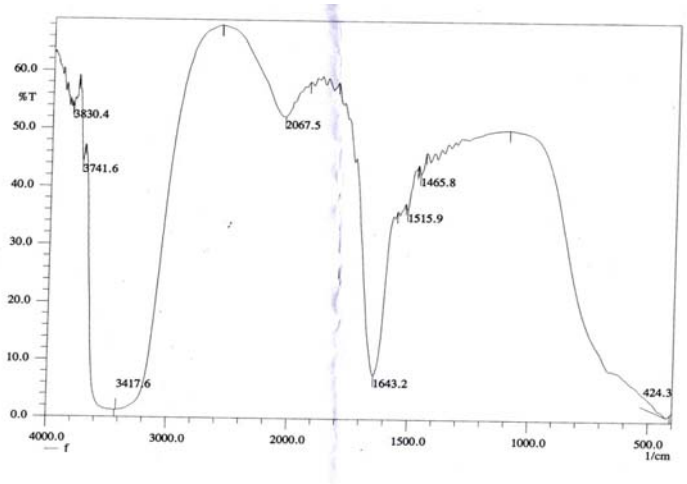

Fig. 5: FTIR spectrum of silver nanoparticles formed by *F. moniliforme*

References

Ahmad A, Mukherjee P, Senapati S, Mandal D, Khan MI, Kumar R, 2003. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. *Colloids Surf. B-Biointerfaces*, **28**: 313-318.

Arun P, Shanmugaraju V, Renga Ramanujam J, Senthil Prabhu S, Kumaran E, 2013. Biosynthesis of Silver Nanoparticles from *Corynebacterium* sp. and its antimicrobial activity. *Int. J. Curr. Microbiol. Appl. Sci.,* **2**: 57-64.

Balaji DS, Basavaraja S, Mahesh BD, Prabhakar BK, Venkataraman A, 2009. Extracellular biosynthesis of functionalized silver nanoparticles by strains of *Cladosporium cladosporioides*. *Colloids Surf. B-Biointerfaces*, **68**: 88-92.

Basavaraja S, Balaji SD, Lagashetty A, Rajasab AH, Venkataraman A, 2008. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium semitectum*. *Mater. Res. Bull.,* **43**: 1164-1170.

Beveridge TJ, Fyfe WS, 1985. Metal fixation by bacterial cell walls. *Can. J. Earth Sci*., **22**: 1893-1898.

Domsch KH, Gams W, 1972. Fungi in agricultural soils. Ongman, London.

Domsch KH, Gams W, Anderson T, 1980. Compendium of soil fungi. Acad. Press. London.

Duran N, Marcato PD, Alves OL, Souzaand G., Esposito E, 2005. Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusarium oxysporum* strains. *J. Nanobiotechnol.,* **8**: 3-8.

Duran N, Marcato P, De Conti R, Alves O, Costa F, Brocchi M, 2010. Potential use of silver nanoparticles on pathogenic bacteria, their toxicity and possible mechanisms of action. *J. Brazil. Chem. Soc*., **21**: 949-959.

Elechiguerra JL, Burt JL, Morones JR, Camacho-Bragado A, Gao X, Lara HH , Yacaman MJ, 2005. Interaction of silver nanoparticles with HIV-1. *J. Nanobiotechnol.*, **3**, 1477-3155.

Fayaz M, Tiwary CS, Kalaichelvan PT, Venkatesan R, 2010. Blue orange light emission frombiogenic synthesized silver nanoparticles using *Trichoderma viride*. *Colloids Surf. B-Biointerfaces*, **75**: 175-178.

Gurunathan S, Lee KJ, Kalishwaralal K, Sheikpranbabu S, Vaidyanathan R, Eom SH, 2009. Antiangiogenic properties of silver nanoparticles. *Biomaterials,* **30**: 6341-6350.

Guzman M, Jean Dille J, Godet S, 2008. Synthesis of silver nanoparticles by chemical reduction method and their antibacterial activity. *Proc. World Acad. Sci. Eng. Technol.,* **2**: 33.

Haefeli C, Franklin C, Hardy K, 1984. Plasmid-determined silver resistance in *Pseudomonas stutzeri* isolated from silver mine. *J. Bacteriol*., **158**: 389-392.

Honary S, Hamed B, Eshrat G, Farzaneh N, 2013. Green synthesis of silver nanoparticles induced by the fungus *Penicillium citrinum*. *Trop. J. Pharm. Res.,* **12**: 7-11.

Huang J, Li Q, Sun D, Lu Y, Su Y, Yang X, Wang H, Wang Y, Shao W, He N, Hong J, Chen C, 2007. Biosynthesis of silver and gold nanoparticles by novel sundried *Cinnamomum camphora* leaf. *Nanotechnology,* **18**: 105104-105115.

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

Ingle A, Mahendra R, Aniket G , Manisha B, 2009. *Fusarium solani*: a novel biological agent for the extracellular synthesis of silver nanoparticles. *J. Nanopart. Res.,* **11**: 2079-2085.

Jaidev LR, Narasimha G, 2010. Fungal mediated biosynthesis of silver nanoparticles, characterization and antimicrobial activity. *Colloids Surf. B-Biointerfaces*, **81**: 430-433.

John FL, Brett AS, 2006. The *Fusarium* laboratory Manual, Blackwell Publishing Ltd.

Jones SA, Bowler PG, Walker M, Parsons D, 2004. Controlling wound bioburden with a novel silver containing hydrofiber dressing. *Wound Repair Regen.*, **12**: 288-294.

Kalishwaralal K, Deepak V, Ramkumarpandian S, Nellaiah H, Sangiliyandi G, 2008. Extracellular biosynthesis of silver nanoparticles by the culture supernatant of *Bacillus licheniformis*. *Mater Lett*., **62**: 4411-4413.

Kim KJ, Sung WS, Suh BK, Moon SK, Choi JS, Kim JG, Lee, DG, 2009. Antifungal activity and mode of action of silver nano-particles on *Candida albicans*. *Biometals*, **22**: 235-242.

Kowshik MS, Ashtaputre S, Khaeeazi W, Vogel J, Urban SK, Kulkarni, Paknikar KM, 2003. Extracellular synthesis of silver nanoparticles. *J. Mat. Chem*., **17**: 2459-2464.

Mandal D, Molander ME, Mukhopadhyay D, Sarkar G, Mukherjee P, 2006. The use of microorganism for the formation of metal nanoparticles and their application. *Appl. Microbiol. Biotechnol.*, **69**: 485-492.

Mann S, Ozin GA, 1996. Synthesis of inorganic materials with complex form. *Nature,* **382**: 313-318.

Minaeian S, Shahverdi AR, Nohi AS, Shahverdi HR, 2008. Extracellular biosynthesis of Silver Nanoparticles by some bacteria. *J. Sci. IAU*., **17:** 1-4.

Mohammed A, Fayaz K, Balaji P, Kalaichelvan T, Venkatesan R, 2009. Fungal based synthesis of silver nanoparticles-an effect of temperature on the size of particles. *Colloids Surf. B-Biointerfaces*, **74**: 123-126.

Nair B, Pradeep T, 2002. Coalescence of nanoclusters and formation of submicron crystallites assisted by *Lactobacillus* strains. *Cryst. Growth Des*., **2**: 293-298.

Nair A, Pradeep T, 2007. Extraction of chlorpyrifos and malathion from water by metal nanoparticles. *J. Nanosci. Nanotechnol*., **7**: 1871-1877.

Narasimha G, Habeeb K, Mohammed A, 2012. Antiviral properties of silver nanoparticles synthesized by *Aspergillus* sp. *Der. Pharmacia Lett.*, **4**: 649-651.

Piksova K, Weiserova M, Jedlickova A, Fojtík A, 2009. Silver nanoparticles and their bactericidal effect. *Nanocon*., **10**: 20-22.

Prashant S, Raja RB, 2011. Biological Synthesis and Characterization of Silver Nanoparticles Using the Fungus *Trichoderma harzianum. Asian J. Exp. Biol. Sci*., **2**: 600-605.

Riddin T, Gericke M, Whiteley C, 2006. Analysis of inter and extracellular formation of platinum nanoparticles by *Fusarium oxysporum* f. sp. *lycopersici* using response surface methodology. *Nanotechnology,* **17**: 3482-3489.

Sanghi R, Verma P, 2009. Biomimetic synthesis and characterization of protein capped silver nanoparticles. *Bioresour. Technol.*, **100**: 501-504.

Sastry M, Ahmad A, Khan MI, Kumar R, 2003. Biosynthesis of metal nanoparticles using fungi and actinomycete. *Curr. Sci.,* **85**: 162-170.

Sheet I, Holail H, Olama Z, Kabbani A, Hines M, 2013.The antibacterial activity of araphite oxide, silver, impregnated graphite oxide with silver and GO-coated sand nanoparticles against Waterborne Pathogenic *E.coli* BL21. *Int. J. Curr. Microbiol. Appl. Sci.,* **2**: 1-11

Shirley A, Dayanand B, Sreedhar G, Syed D, 2010. Antimicrobial activity of silver nanoparticles synthesized from novel *Streptomyces* species. *Dig. J. Nanomater. Bios*., **5**: 447-451.

Singaravelu G, Arockiamary JS, Kumar VG, Govindaraju K, 2007. A novel extracellular synthesis of monodisperse gold nanoparticles using marine alga, *Sargassum wightii* Greville. *Colloids Surf. B-Biointerfaces*, **57**: 97-101

Sondi I, Sondi BS, 2004. Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria. *J. Colloid. Inter. Sci*., **275**: 177-182.

Velmurugan N, Kumar GG, Han SS, Nahm SK, Lee SY, 2009. Synthesis and characterization of potential fungicidal silver nano-sized particles and chitosan membrane containing silver particles. *Iran. Polym. J.,* **18**: 383-392.

Vighneshwaan N, Kathe, AA, Vardadarajan PV, Nbachane, RP, Bala Subramanian RH, 2007. *Langmuir*, **23**: 7113-7117.

Yan Z, Ying K, Subrata D, and Hong L, 2012. Antibacterial activities of gold and silver nanoparticles against *Escherichia coli* and *Bacillus*. *J. Nanobiotechnol*., **10**: 19-28.

Zhang H, Li Q, Lu Y, Sun D, Lin X, Deng X, 2005. Biosorption and bioreduction of diamine silver complex by *Corynebacterium*. *J. Chem. Technol. Biotechnol*., **80**: 285-290.