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Research Article

ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES SYNTHESIZED FROM *FUSARIUM SEMITECTUM*

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ABSTRACT
The development of reliable process for synthesis of silver nanomaterials is an important aspect of nanotechnology today. In the present work an attempt was made for extra cellular biosynthesis of silver nanoparticles using culture supernatant of *Fusarium semitectum***.** The filtrate of *F. semitectum* biomass with silver ions was observed at the beginning and after two days of reaction. The color of the solution turns from colorless to brown indicating the formation of silver nanoparticles. The formation of nanoparticles was monitored by UV-visible spectra. The synthesized silver nanoparticles were tested against bacterial cultures and in treatment of waste water and found to have anti bacterial activity.

Keywords: *Silver Nanoparticles, Fusarium Semitectum, Antibacterial Activity, Waste Water*

INTRODUCTION
Nanoparticles are regarded as highly reactive species because of large surface area. The most effectively studied nanoparticles today are those made from noble metals, in particular Ag, Pt, Au, Pd (Arangasamy and Vivekananda, 2008). Among metal nanoparticles, silver nanoparticles play a significant role in the field of biology and medicine. The silver nanoparticles have been tested in various fields of biological science viz. drug delivery, wound treatment , binding of HIV gp 120 protein (Elchiguuerra *et al.,* 2005), in water treatment and as an antibacterial compound (Baker *et al.,* 2005; Jain and Pradeep, 2005).
The silver nanoparticles have been synthesized using a variety of methods (Duran *et al.,* 2005; Mouxing *et al.,* 2006; Sun *et al.,* 2003). Among various methods, biological method is considered as an eco-friendly. With the increasing demand for green synthesis processes, the field of nanoparticle synthesis employed either biological microorganisms or plant extracts as a simple and viable alternative to chemical procedures and physical methods (Arangasamy and Vivekananda, 2008; Minaeian *et al.,* 2008; Sastry *et al.,* 2003). Various microorganisms used for the production of silver nanoparticles include Pseudomonas stutzeri, Klebsiella pneumoniae, Escherishia coli, Enterobacter cloacae (Klaus-Joerger *et al.,* 2001; Minaeian *et al.,* 2008.).
The fungi are the most suitable organisms for biosynthesis of nanoparticles as their metabolic activity lead to precipitation of nanoparticles in external environment. A method for the synthesis of nanoparticles of gold (Mukherjee *et al.,* 2001a) and silver (Mukherjee *et al.,* 2001b) intracellularly in Verticillium fungal cells was reported. The intracellular synthesis of nanoparticles may accomplish a better control over the size and shape distribution of the product, harvesting and recovery are more cumbersome and expensive. The extra cellular synthesis by comparison is more adoptable. Some researchers found that the silver nanoparticles can be synthesized extracellularly by using fungi Colletotrichum sp., (Mandal, 2006), Aspergillus fumigatus (Bhainsa, 2006), Fusarium oxysporum (Ahmad *et al.,* 2003) and Fusarium semitectum (Basavaraja *et al.,* 2008). The present study is an attempt to synthesize silver nanoparticles extracellularly from F. semitectum and to assess the antibacterial activity of these nanoparticles.

MATERIALS AND METHODS
Synthesis of silver nanoparticles from F. semitectum
The fungus *F. semitectum* was obtained from Agarkar Research Institute, Pune, India and maintained on Potato Dextrose Agar slants. The procedure for biosynthesis of silver nanoparticles was followed as described by Basavaraj *et al.,* (2008). To prepare the biomass for biosynthesis studies, the fungus was grown aerobically in a liquid media containing (g/l) of KH_2PO_4, 7.0; K_2HPO_4, 2.0; $MgSO_4$, 7; H_2O, 0.1;

$(NH_2)SO_4$, 1.0; Yeast extract, 0.6; and glucose, 10.0. After 72 hr of growth, 20 g (wet wt.) of biomass was taken and in 100 ml of double distilled water and agitated for 72 h at 27 ^{0}C in Erlenmeyer flask. The filtrate obtained was then mixed with 100 ml of 10^{-3} M $AgNO_3$ solution in 250 ml Erlenmeyer flask and kept on shaker at 27 ^{0}C. Periodically aliquots of the reaction solution were removed and subjected to UV-Vis spectroscopy measurement at resolution of 1 nm from 200-800 nm.

Microorganisms

Standard cultures of following microorganisms were maintained by sub culturing at regular intervals in nutrient agar medium.

Gram positive bacteria: *Staphylococcus aureus, Streptococcus pyogens*

Gram negative bacteria: *Pseudomonas aeruginosa, Salmonella typhi*

Determination of Zone of Inhibition by Cup Plate Method

The antibacterial activity of silver nanoparticles was performed using agar cup plate method (Pelczar and Reid, 1974). Sterile nutrient agar medium (20ml) was poured in to sterile petri dishes and allowed to solidify. The medium was seeded with the organism by pour plate method. Bores were made on the medium with sterile cork borer. Different concentrations (50, 100 and 150 µl) of silver nanoparticles were added to respective bores. Penicillin (1mg/ml) was used as standard reference. Petri dishes were kept in refrigerator at 4 ^{0}C for 30 min. for diffusion. After diffusion the petri plates were incubated at 37 ^{0}C for 24 h and zones of inhibition were observed and measured.

Silver Nanoparticles in Waste Water Treatment:

Antibacterial effect was assessed against the bacteria present in sewage and waste water samples by spread plate method. By employing serial dilution technique, the bacteria were isolated from samples before treating with silver nanoparticles and CFU were recorded. Then 1ml of sample was treated with different concentrations (0.5, 1 and 2 ml) of silver nanoparticles for varying time intervals (3, 4 and 5 hr) and both the sets were grown on petri plates containing 20 ml of nutrient agar medium. The plates were incubated at 37 ^{0}C for 24 hrs and colony forming units (CFU) were counted after 24 hrs.

RESULTS AND DISCUSSION

Extracellular Biosynthesis of Silver Nanoparticles from F. Semitectum

The development of reliable process for the synthesis of silver nanomaterials is an important aspect of nanotechnology today. In the present work, the extracellular biosynthesis of silver nanoparticles was carried out by using culture supernatant of *F. semitectum*. The Erlenmeyer flasks with the fungal filtrate has a pale yellow color before the addition of Ag+ ions which changed to a brownish color on completion of the reaction with silver ions for 24 h. The appearance of brownish color in solution containing the biomass is a clear indication of the formation of silver nanoparticles in the reaction mixture (Duran *et al.*, 2005). Our results are in agreement with that of Basavaraj *et al.,* (2008) and Saifuddin *et al.,* (2009), who also employed *F. semitectum* for the synthesis of silver nanoparticles. The change in color is known to arise owing to the surface plasmon resonance of metal nanoparticles (Sastry *et al.,* 2003). The UV-Visual spectra (Fig 1.) recorded for the aqueous silver nitrate-*F. semitectum* reaction medium as a function of time indicated that the silver surface plasmon band occurs at around 425 nm and steadily increased in intensity.

Antibacterial Activity of Silver Nanoparticles

The silver nanoparticles are the metal of choice as they hold the promise to kill microbes effectively (Sondi and Sondi, 2004). In the present study, the silver nanoparticles synthesized from F. semitectum were evaluated for antibacterial activity against two Gram positive and two Gram negative bacteria of high pathogenic nature. The results (Table 1) showed that the silver nanoparticles at the concentration of 150 µl have shown maximum zone of inhibition compared to 50 µl and 100 µl. The zone of inhibition was 50 % in case of *P. aeruginosa* and *S. aureus* and 85.7% in case of *S. pyogens* when compared to standard antibiotic penicillin. The bacterium *S. typhi* was found to be more resistant to silver nanoparticles as the zone of inhibition was only 34.5%.

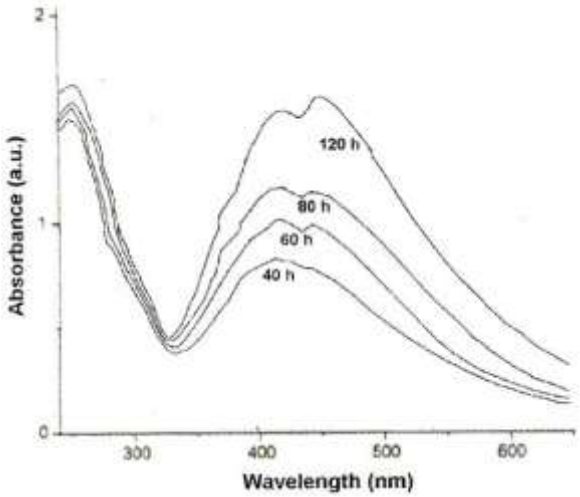

Figure 1: UV Visible absorption spectra recorded as a function of time of reaction at 10^{-3} M aqueous solution of silver nitrate with the fungal biomass. The time of reaction is indicated next to the respective curves

Dharmendra *et al.,* (2008) have studied the antibacterial effect of silver nanoparticles synthesized by a physical, top-down approach technique against *E. coli* and *B. subtlis*. They found that the effect is time, dose and strain dependent. They also reported that the silver nanoparticles cause antibacterial effect by rupturing cell membrane. In the present study also, the antibacterial activity increased with increased concentration of silver nanoparticles. Silver nanoparticles are reported to have antibacterial effect against *Klebsiella pneumoniae* and *S. aureus* (Duran *et al.*, 2007; Jain and Pradeep, 2005; Sons *et al.*, 2004) when used alone and effective against *E. coli, B. subtilis* 1021, *P. syringae pv. syringae* 2440, *Xanthomonas compestris pv. vesicatoria, Azotobacter chrococcum* SL 206 and *Rhizobium tropici* when used in combination with silica (Hae-Jun *et al.*, 2006). To enhance the antibacterial effect of silver nanoparticles, they are also combined with antibiotics (Ping *et al.,* 2005) or Ultrasonic irradiation (Dharmendra *et al.,* 2008).

Table 1: Antibacterial Effect of Silver Nanoparticles Against Selected Bacteria

Bacteria	***Penicillin 1mg/ml***	***Concentration of silver nanoparticles***		
		50 µl	100 µl	150 µl
Staphylococcus aureus	28	09	10	14
Streptococcus pyogens	14	06	08	12
Salmonella typhi	29	06	07	10
Pseudomonas aeruginosa	22	08	10	11

Values indicate the zone of inhibition in mm

Silver nanoparticles in waste water treatment

The removal or inactivation of pathogenic microorganisms is the last step in the treatment of waste water. Research is under way to use advances in nanotechnology in water purification. Silver nanoparticles have been used as antimicrobial compounds for coliform found in waste water (Jain and Pradeep, 2005). In the present study, the CFU of bacteria before treating with silver nanoparticles were 312 in sewage and 201 in waste water. The silver nanoparticles reduced the CFU of bacteria from 312 to 17 and 201 to 12 in sewage and waste water respectively at a concentration of 1.5ml and a time interval of 5hr (Table 2). This effect is found to be concentration and time dependent. A study made by Dharmendra *et al.,* (2008) on the combined antibacterial effect of silver nanoparticles and ultrasonic irradiation on E. coli cells isolated from waste water reduced the number of colonies to very few after 35 minutes of treatment of silver nano

-particles at a concentration of 10^{21} molecules /cc.

Table 2: Antibacterial Effect of Silver Nanoparticles against Sewage and Waste Water

Sl. No.	Time interval (h)	Concentration of silver nanoparticles		
		0.5 ml	1.0 ml	1.5 ml
Sewage water				
1	3	250	99	47
2	4	140	39	21
3	5	95	30	17
Waste water				
1	3	145	95	32
2	4	120	37	15
3	5	93	28	12

Values indicate number of colony forming units (CFU)

REFERENCES

Ahmad A, Mukherjee P, Senapati S, Mandal D, Islam Khan M, Rajiv K and Sastry M (2003). Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum. Colloids and Surfaces B: Biointerfaces* **28** 313-316.

Arangasamy L and Munusamy V (2008). Tapping the unexploited plant resources for the synthesis of silver nanoparticles. *African Journal of Biotechnology* **7** 3162-3165.

Baker C, Pradeep A, Pakstis L, Pochan DJ and Shah SI (2005). Synthesis and antibacterial properties of silver nanoparticles. *Journal of Nanoscience and Nanotechnology* **5** 244-249.

Basavaraja S, Balaji SD, Arunkumar L, Rajasab AH and Venkataraman A (2008). Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium semitectum*. *Materials Research Bulletin* **43** 1164-1170.

Bhainsa KC and D' Souza SF (2006). Extracellular biosynthesis of silver nanoparticles using the fungus Aspergillus fumigatus. *Colloids and Surfaces B: Biointerfaces* **47** 160-164.

Dharmendra KT, Behari J and Sen P (2006). Time and dose dependent antimicrobial potential of Ag nanoparticles synthesized by top-down approach. *Current Science* **95** 647-655.

Dharmendra KT, Behari J and Sen P (2008). Application of nanoparticles in waste water treatment. *World Applied Sciences Journal* **3** 417-433.

Duran N, Marcato PD, Gabriel IH, De Souza, Oswaldo LA and Eliza E (2007). Antibacterial effect of silver nanoparticles produced by fungal process on textile fabrics and their effluent treatment. *Journal of Biomedical Nanotechnology* **3** 203-208.

Elchiguuerra JL, Burli JL, Morones JR, Camacho-Bragado A, Gao X, Lara HH and Yacaman MJ (2005). Interaction of silver nanoparticles with HIV I. *Journal of Nanobiotechnology* **3** 1477-3155.

Hae-Jun P, Sung HK, Hwa Jung K and Seong HC (2006). A new composition of nanosized silica-silver for control of various plant diseases. *Journal of Plant Pathology* **22** 295-302.

Jain P and Pradeep T (2005). Potential of silver nanoparticle coated polyurethane foam as an antibacterial water filter. *Biotechnology and Bioengineering* **90** 59-63.

Klaus-Joerger T, Joerger R, Olsson E and Granqvist C (2001). Bacteria as workers in the living factory: Metal-accumulating bacteria and their potential for materials science. *Trends in Biotechnology* **19** 15-20.

Mandal D, Bolander ME, Mukhopadhyay D, Sarkar G and Mukherjee P (2006). The use of most for the formation of metal nanoparticles and their application. *Applied Microbiology and Biotechnology* **69** 485-492.

Minneian S, ShahverdiAR, Nohi AS and Shahvardi HR (2008). Extracellular biosynthesis of silver nanoparticles by some bacteria. *Journal of Sciences (Islamic Azad University, winter)* **17**(66/1 (Biology Issue)) 1-4.

Mouxing F, Qingbiao L, Daohua S, Yingucha L, Ning H, Xu D, Huixuan W and Jiale H (2005). Rapid preparation process of silver nanoparticles by bioreduction and their characterization. *Chinese Journal of Chemical Engineering* **14** 114-117.

Mukherjee P, Ahmad A, Mandal D, Senapati S, Sainkar SR, Khan MI, Parisha R, Ajaykumar PV, Alam M, Rajiv K and Sastry M (2001b). Fungus mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: A novel biological approach to nanoparticle synthesis. *Nano Letters* 515.

Mukherjee P, Ahmed A, Mandal D, Senapati S, Sainkar SR, Khan MI, Ramani R, Parisha R, Ajaykumar PV, Alam M, Sastry M and Rajiv K (2001a). Bioreduction of $AuCl^{+}$ ions by the fungus *Verticillium* sp. and surface trapping of the gold nanoparticles formed. *Angewandte Chemie International Edition* **40** 3585.

Pelczar MJ and Reid JD (1974). *Microbiology* (Tata Mcgraw Hill, New Delhi) 473.

Ping Li, Li J, Wu C and Li J (2005). Synergistic antibacterial effect of B- Lactum antibiotic combined with silver nanoparticle. *Nanobiotechnology* **16** 1912-1917.

Saifuddin N, Wong CW and Yasumira AAN (2009). Rapid biosynthesis of silver nanoparticles using culture supernatant of bacteria with microwave irradiation. *E-Journal of Chemistry* **6** 61-70.

Sastry M, Absar A, Islam Khan M and Rajiv K (2003). Biosynthesis of metal nanoparticles using fungi and actinomycete. *Current Science* **2** 162-170.

Sondi I and Sondi BS (2004). Silver nanoparticles as antimicrobial agent: a case study on *E.coli* as a model for Gram negative bacteria. *Journal of Colloid and Interface Science* **275** 177-185.

Sons WK, Youk JH, Lee TS and Park WH (2004). Preparation of antimicrobial ultrafine cellulose acetate fibres with silver nanoparticles. *Macromolecular Rapid Communications* **22** 295-302.

Sun Y, Mayers B, Herricks T and Xin Y (2003). Polyol synthesis of uniform silver nanowires: A plausible growth mechanism and the supporting evidence. *Nano Letters* 955-960.