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Fusarium oxysporum Mediates Photogeneration of Silver Nanoparticles

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In this study, 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 5-60 nm in dimension. The nanoparticles were examined using UV-Visible Spectroscopy, Scanning Electron Microscopy (SEM), Energy Dispersive X-ray Spectroscopy (EDS) and Transmission Electron Microscopy (TEM) analyses. The formation of nanoparticles by this method is extremely rapid, undertaken in ambient conditions and the synthesized hydrosol is stable for several months in the absence of light.

INTRODUCTION

There is little doubt that nanomaterials will play a key role in many technologies of the future. This is mainly due to the possibility of a drastic scale down in all technologies while improving their performance at the same time. In addition, nanomaterials have unique physico-chemical and optoelectronic properties, due to the quantum confinement phenomenon (i.e., confinement of electrons within particles with dimensions smaller than the bulk electron delocalization length) and, so, furnish an unexplored pool of features for the scientist.

An industrially important sector of nanoscience deals with the preparation and study of nanoparticles. Silver nanoparticles have already been used in antibacterial clothing and burn ointments and as coating for medical devices, because of their mutation-resistant anti-microbial activity [1]. They have also been used in catalysis [2] and biosensors [3].

With the flourishing demand of "green" nanoparticle synthesis processes [4], the field of nanoparticle synthesis has recently developed new routes for nanoparticles. These include employing microorganisms, such as: *Pseudomonas stutzeri* [5] *Verticilium sp.* [6], *Fusarium oxysporum* [7,8], *Thermomonospora sp.* [9] and, also, alfalfa [10] and geranium [11] plants. The production of extremely stable silver hydrosols, using the fungus *Fusarium oxysporum*, has previously been reported by Ahmad et al. [7]. Despite stability and a green method of production, particles are not monodispersible and the rate at which they form is not comparable to chemical synthesis methods. This calls for attempts both to find new organisms and to improve methods.

Here, the first photobiological approach to generate silver nanoparticles is reported, which opens up new horizons in the combinatorial synthesis of nanoparticles. The method used in this paper leads to the formation of nanoparticles in less than 1 hour, compared to 96 hours reported previously using the same fungus [8].

MATERIALS AND METHODS

Various strains of *Fusarium oxysporum* were tested for the synthesis of silver nanoparticles. Strain 5115 from the Persian Type Culture Collection (PTCC) and strains 23, 24, 25 and 30, from the National Research Center for Genetic Engineering and Biotechnology (NRCGEB) (a gift granted by Dr. Zamani) [12] were cultured on Vogel's medium [13] with either sucrose or glucose as the carbon source. The choice of the defined medium was made to ensure the absence of (varying amounts of) unknown components found in the complex media used by Ahmad et al. [8] and to attain better reproducibility. All chemicals of the culture media were obtained from Merck, Germany.

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After 96 hours of incubation of the fungus at 25°C, with shaking at 120 rpm, the biomass was centrifuged out at 6500 g for 10 minutes. A fresh solution of 10^{-2} M silver nitrate (Aldrich chemicals) was added to the supernatant to yield an overall Ag^+ ion concentration of 10^{-3} M in the resulting solution.

The reaction was carried out under a conventional halogen-tungsten lamp, which provided 90,000 lux (measured by a Lx103 light meter, Lutron) at the surface of the reaction vessel. The reaction vessel was a cylinder with a working volume of $\pi\times$ (1.25 cm)^2 $\times$ 1 cm. Control experiments were conducted with un-inoculated media, to check for the role of fungus in the synthesis of nanoparticles.

The spectrum of the reacting solution was taken several times during the course of the reaction by a Cary 50 spectrophotometer, in a 1 cm path quartz cell. A representative nanoparticle solution was mounted on formvar coated grids and subjected to TEM study on a Zeiss 902A (Germany) operating at 80 kV. The size distribution study was obtained manually by measuring the dimensions of 383 particles in scanned TEM micrographs. The SEM and EDS studies were done at 20 kV using a Philips XL 30, the Netherlands.

A full factorial design was devised to investigate the best conditions for the synthesis of nanoparticles in the ambient condition. The details of experimental conditions are listed in Table 1.

Table 1. Full factorial design to find the optimum condition for the growth of fungus. The response is listed in the last column.

Done Order	Standard Order	Sucrose (g/l)	Shaker (rpm)	T (°C)	Area (100 nm. Absorption units)
1	15	17	120	28	2.182
2	11	17	120	24	2.289
3	9	13	120	24	2.278
4	8	17	200	28	2.324
5	16	17	200	28	2.264
6	6	13	200	28	2.332
7	13	13	120	28	2.270
8	14	13	200	28	2.262
9	4	17	200	24	2.221
10	7	17	120	28	2.211
11	3	17	120	24	2.209
12	12	17	200	24	2.301
13	1	13	120	24	2.207
14	5	13	120	28	2.210
15	10	13	200	24	2.260
16	2	13	200	24	2.221

RESULTS AND DISCUSSION

The production of silver nanoparticles was not observed in un-inoculated media. Relative rates of nanoparticle synthesis (measured as the rate of increase in the plasmon peak area) for different strains are listed in Table 2. The various spectra, recorded at different times during the reaction course for strain 25, are presented in Figure 1. These spectra, as evidenced by the peak width, suggest a degree of agglomeration not much different from the results obtained in 96 hours by Ahmad et al. [7]. The plasmon peaks are located at wavelengths of about 440 nm, suggesting the presence of rather large metallic silver nanoparticles [14]. Representative TEM and SEM micrographs, with different magnifications from the hydrosols, are shown in Figures 2 and 3. The EDS of a nanoparticle verifies the particle is, indeed, metallic silver (Figure 2C). Nanoparticle size distribution, obtained from 383 particles, is shown in Figure 4. The results of the full factorial design are presented in the right column of Table 1. It should be noted that, although the fungus can grow outside the temperature range tested here, the temperature range at around room temperature is used to maintain a "green" procedure. The statistical interpretation of factorial design reveals that the rate of synthesis of silver nanoparticles shows little

Table 2. Relative rates of development of plasmon peaks in reaction vessel for different strains of fungus.

Strain	Development of Plasmon Peak Area
23	0.769
24	0.798
25	1.000
30	0.374
5115	2.002

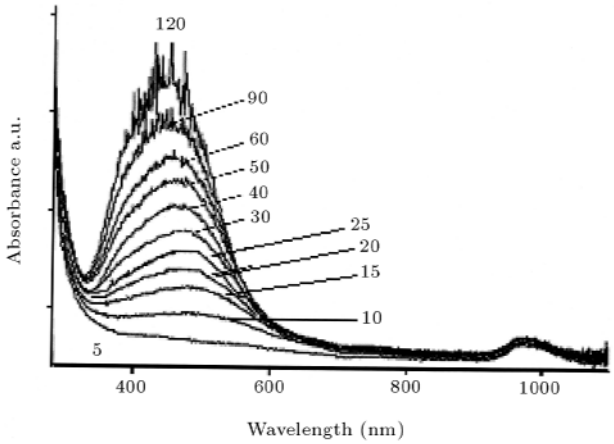

Figure 1. Time dependent absorbance spectra of nanoparticle solutions. The time at which the spectrum was recorded is written in minutes.

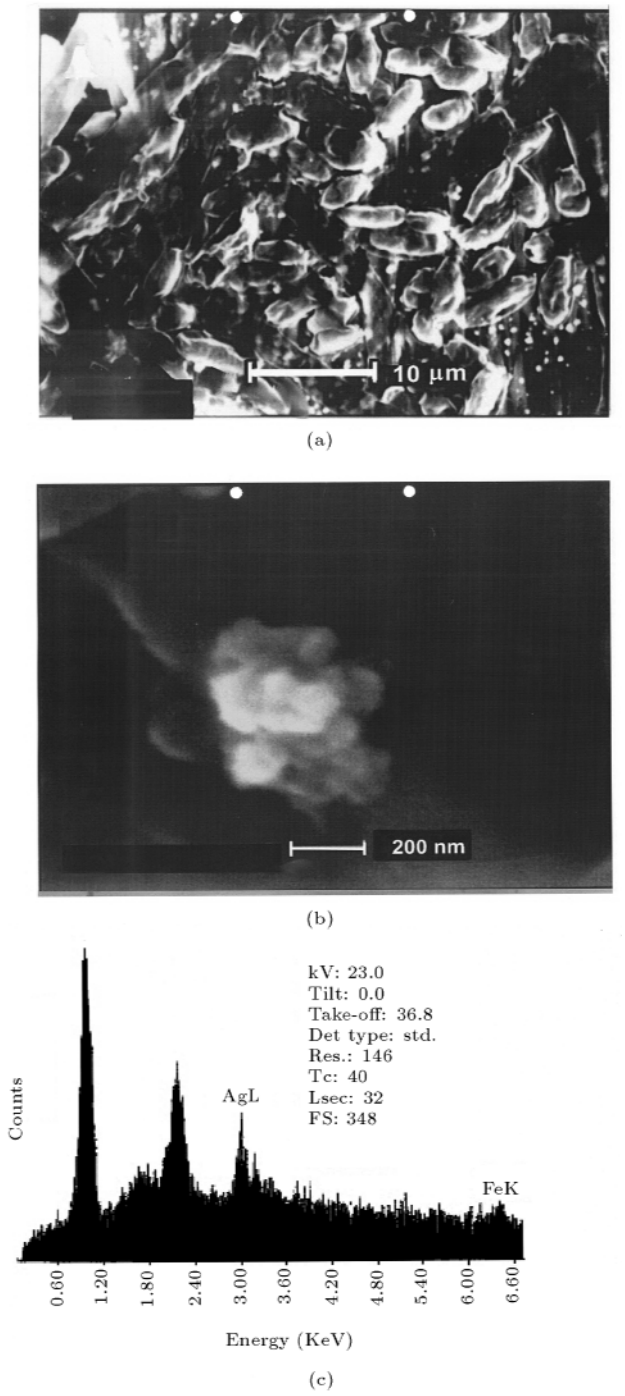

Figure 2. SEM images of the nanoparticles: a) With the fungal biomass (nanoparticles seen in lower right part of the figure); b) A single nanoparticle. Note that it is an agglomeration of smaller particles with an agglomeration number much less than that seen in previous reports; c) EDS analysis of the particle in Figure 2b.

dependency on the conditions applied (no significant parameter with 95% confidence). This result suggests that the consistency in the fungus growth conditions (temperature of growth, concentration of carbon source and shaking frequency) is not a determining factor on

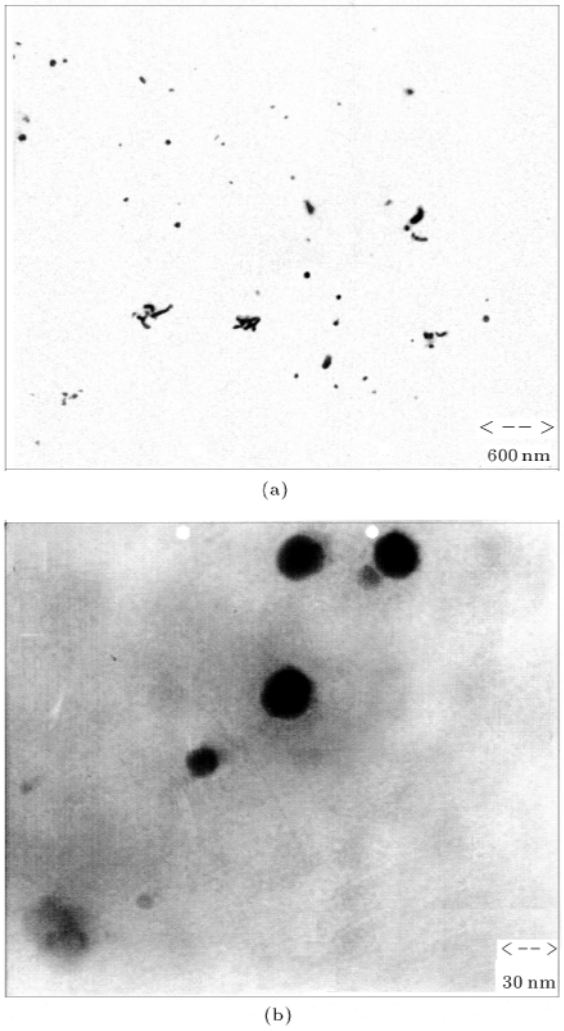

Figure 3. Transmission electron microscopy micrographs of nanoparticles; a) 12000 x and b) 50000 x.

the rate of nanoparticle synthesis with stationary-phase fungus.

Understanding the nature of capping agents conferring stability to the nanoparticles obtained by this method, is quite important, as the method produces an extremely stable nanoparticle solution. The stability of nanoparticles is attributed to the action of capping agents. An understanding of the species acting as capping agents can be obtained by subjecting the culture media to chromatography, exposing different portions of the media to the nanoparticles capped by other known materials (such as citrate) to have a ligand exchange, followed by an FTIR. This would be routine work, as one needs to consider just the extracellular components secreted by the fungus to the medium and, so, facing few chemicals or biological molecules.

CONCLUSION

This work clearly shows the possibility of enhancement in the application of microorganisms to the synthesis

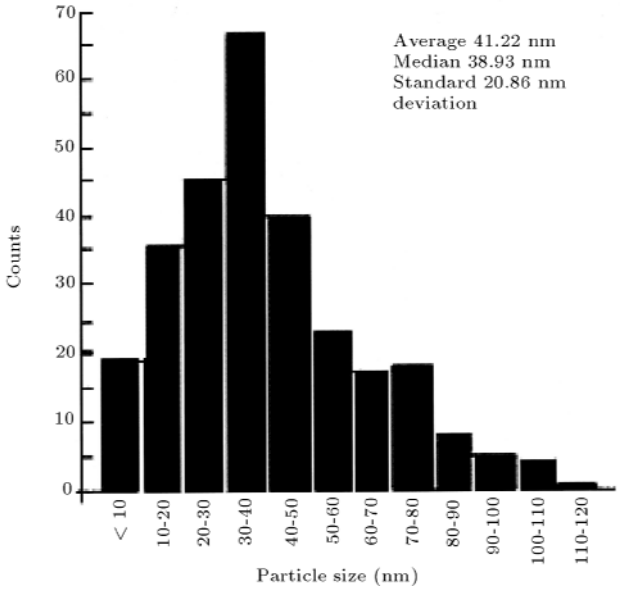

Figure 4. Particle size distribution as obtained from 383 nanoparticles. Average nanoparticle size as well as standard deviation in nanoparticle diameter is given in nanometers.

of nanoparticles. It is shown that the rate of formation of nanoparticles can compete with chemical routes. Furthermore, by comparison of SEM micrographs, it becomes evident that the degree of agglomeration of nanoparticles in this study is far less than that reported by Durán et al. [15].

There is also the possibility of genetically engineering microbes to over-express specific reducing molecules and capping agents and, thereby, controlling the size and shape of the biogenic nanoparticles.

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REFERENCES

1. Deitch, E.A., Marrino, A.A., Lakanok, V. and Albright, J.A. "Silver nylon cloth: In vitro and in vivo evaluation of antimicrobial activity", *J. Trauma*, **27**(3), pp 301-304 (1987).
2. Weber, A.P., Seipenbusch, M. and Kasper, G. "Size effects in the catalytic activity of unsupported metallic nanoparticles", *J. Nanoparticle Res.*, **5**(1), pp 293-298 (2003).
3. Haes, A.J. and Van Duyne, R.P. "A nanoscale optical biosensor: Sensitivity and selectivity of an approach based on the localized surface plasmon resonance spectroscopy of triangular silver nanoparticles", *J. Am. Chem. Soc.*, **124**, pp 10596-10604 (2002).
4. Huang, H. and Yang, X. "Synthesis of polysaccharide-stabilized gold and silver nanoparticles: A green method", *Carbohydr. Res.*, **339**, pp 2627-2631 (2004).
5. Klaus-Joerger, T., Joerger, R., Olsson, E. and Granqvist, C. "Bacteria as workers in the living factory: Metal-accumulating bacteria and their potential for materials science", *Trends Biotechnol.*, **19**(1), pp 15-20 (2001).
6. Mukherjee, P., Ahmad, A., Manda, l.D., Senapati, S., Sainkar, S.R., Khan, M.I., Parishcha, R., Ajaykumar, P.V., Alam, M., Kumar, R. and Sastry, M. "Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: A novel biological approach to nanoparticle synthesis", *Nano Lett.*, **1**(10), pp 515-519 (2001).
7. Ahmad, A., Mukherjee, P., Senapati, S., Mandal, D., Khan, M.I., Kumar, R. and Sastry, M. "Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*", *Coll. Surf. B*, **28**(4), pp 313-318 (2003).
8. Mukherjee, P., Senapati, S., Mandal, D., Ahmad, A., Islam Khan, M., Kumar, R. and Sastry, M. "Extracellular synthesis of gold nanoparticles by the fungus *Fusarium oxysporum*", *Chem. Bio. Chem.*, **3**, pp 461-463 (2002).
9. Ahmad, A., Senapati, S., Islam Khan, M., Kumar, R. and Sastry, M. "Extracellular biosynthesis of monodisperse gold nanoparticles by a novel *Extremophilic actinomycete, Thermomonospora sp.*" *Langmuir*, **19**, pp 3550-3553 (2003).
10. Gardea-Torresdey, J.L., Gomez, E., Peralta-Videa, J.R., Parsons, J.G., Troiani, H. and Yacaman, M.J. "Alfalfa sprouts: A natural source for the synthesis of silver nanoparticles", *Langmuir*, **19**, pp 1357-1361 (2003).
11. Shankar, S.S., Ahmad, A. and Sastry, M. "Geranium leaf assisted biosynthesis of silver nanoparticles", *Biotechnol. Prog.*, **19**, pp 1627-1631 (2003).
12. Motallebi, M., Zamani, M.R., Jazayeri, O. and Harighi, M.J. "Use of RAPD, enzyme activity staining, and colony size to differentiate phytopathogenic *Fusarium oxysporum* isolates from Iran", *Brazil. J. Microbiol.*, **33**, pp 1-5 (2002).
13. Vogel, H.J. "A convenient growth medium for *Neurospora*", *Microbial Genetics Bulletin*, **13**, pp 42-43 (1956).
14. Mulvaney, P. "Surface plasmon spectroscopy of nanosized metal particles", *Langmuir*, **12**, pp 788-800 (1996).
15. Durán, N., Marcato, P.D., Alves, O.L., De Souza, G.I.H. and Esposito, E. "Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusarium oxysporum* strains", *J. Nanobiotechnology*, **3**, pp 8-14 (2005).