

# Biologically synthesised Silver nanoparticles using a Bacterial Culture Isolated from the riverine bank of Ganges in India

Naheed Ahmad\*, Seema Sharma\*\* and Ashok K Ghosh\*\*\*

\* University Department of Botany, Patna University, Patna 800003  
naheedshamsi@yahoo.co.in

\*\*Department of Physics, A N College, Patna 800013  
seema\_sharma26@yahoo.com

\*\*\* Department of Environment and Water Management, A N. College, Patna 800013  
ashokghosh51@hotmail.com

## ABSTRACT

Nanotechnology is a highly energized discipline of science and technology. One of the major challenges of nanotechnology is the synthesis of nanomaterials with a wide range of chemical compositions and sizes. Nanobiotechnology is a promising novel field specially for biodiverse countries like India whose diversity is an asset which can be harnessed. Recently material scientists have been viewing with interest this diversity, particularly microorganisms as possible ecofriendly nanofactories..

In this paper, biosynthesis of nanomaterials is carried out from the microbial diversity obtained from the state of India, Bihar. *Bacillus* species isolated locally from the region has been used to synthesize silver nanoparticles at room temperature.

## KEYWORDS

nanobiotechnology, nanofactories, microbial diversity, biosynthesis

## INTRODUCTION

Nanotechnology is a highly energized discipline of science and technology, which is gaining importance in the new millennium. Nanomaterials are of considerable interest due to their unusual optical [1], chemical [2], petrochemical [3] and electronic properties [4]. These nanoscale materials have gained importance due to their potential applications in optics, biomedical sciences, mechanics, magnetism and energy science. Hence one of the various challenges of nanotechnology is the synthesis of nanomaterials with a wide range of chemical composition and sizes. In the current

world there is a growing need and awareness to develop green technologies.

Biological systems, are in general masters of ambient condition of chemistry and hence have ability to synthesize and sequester inorganic materials from nano to macroscale. Recently material scientists have been viewing with interest biological systems particularly microorganisms as possible

ecofriendly nanofactories [5-7]. Nanobiotechnology is a promising field specially for biodiversity rich countries like India [8]. Biodiversity, though the planet's greatest asset remains paradoxically the least developed [9]. Microorganisms occupy diverse habitats, they develop unique and sometimes bizarre ways to survive hostile toxic environments. Metals are ubiquitous and many are necessary for the survival of organisms but even essential elements like Fe, Zn, Mg, etc. are toxic to organisms beyond certain limits. Bacteria react to most metals by reduction of ions or by the formation of water insoluble complexes. Microorganisms that tolerate heavy exposure to metals can be used for synthesis of nanoscale materials. However the exact mechanism leading to the formation of nanoparticles is not fully understood. Formation can be either intracellular or extra cellular. The ability of microorganisms to grow in presence of high metal concentrations might be a result of specific mechanisms of resistance. Such mechanisms include efflux systems, alteration of solubility and change of toxicity in redox state of the metal ion, extracellular complexation and lack of specific metal transport systems [10-11].

Early studies have revealed the use of various microorganisms for nanosynthesis. Bacteria *Bacillus subtilis* 168 was reported to reduce Au<sup>+</sup> to nanoscale dimensions [6,7]. Several bacterial strains like *Pseudomonas stutzeri* Ag259 were found to be silver resistant and were able to produce nanosize silver [12]. Other workers have used the microbial diversity to produce Magnetite, Silica, Titania from fungi *Fusarium oxysporum* and *Vericillium* [13-14]. *Klebsiella aerogens* was manipulated to produce Cds

nanoparticles extracellularly[15]. In addition to gold and silver nanoparticles synthesis of semiconductors like CdS , ZnS and PbS have been obtained from bioorganisms *Clostridium thermoaceticum* precipitates was observed to precipitate CdS at the cell surface as well as the medium from CdCl<sub>2</sub> in presence of cysteine hydrochloride in the growth media [16].The monodispersity of silver / gold nanoparticles produced either intra or extracellularly by these bioorganisms is not very high or inferior to those obtained by the conventional chemical methods[17]

In this paper, biosynthesis of nanomaterials is carried out from the microbial diversity obtained from the state of India, Bihar (rich fertile alluvial Gangetic plain).*Bacillus* species which was identified as *Bacillus cresus* has been used to synthesize silver nanoparticles which was isolated from the Gangetic belt (riverine belt of Ganges river) was seen to synthesize silver nanoparticles extracellularly.This is the first report when the microbial diversity of the Gangetic plain has been tapped for nanosynthesis.

### METHODOLOGY

Soil samples were collected from the riverine belt of Ganges in Bihar . Serial dilutions were done and plated on N.A plates. The plates were incubated for suitable time and distinct looking colonies were picked up and grown till pure cultures were obtained . Preliminary Silver resistance of various strains was done . The cultures were screened by spot inoculation of Silver nitrate of varying concentrations. The concentrations used were 25ppm .50 ppm ,75ppm , 100ppm . 150 ppm 200ppm 250ppm, 300 ppm ...600ppm 800ppm till 1000ppm . Strains which could tolerate 800ppm of silver was selected for further study .

The selected silver tolerant strain was inoculated in YES media and incubated at room temperature until the absorbance of the culture was between 0.8-0.9 . silver at a concentration of 800 ppm was added and cultures were incubated further . Cell free supernatant was obtained by centrifugation of the cultures at 8000rpm for 10 min at room temperature.The absorbance scan of the supernatant was recorded to check for the characteristic nano silver peak. Cultures which showed best peak were selected for further studies. Optimization of the cultures was done to get the maximum yield.

Optical absorbance of the silver nanoparticles was taken on UV visible spectrophotometer (Shimadzu UV 2450), in range of 200-800 nm .XRD measurements of silver nanoparticles was done on Rigaku Miniflex and TEM of the silver nanoparticles was performed on carbon coated copper grids .

### RESULTS AND DISCUSSION

The results revealed that the soil sample contains a diversity of unique bacteria which are silver tolerant. The bacterial culture which was selected for further study was identified as *Bacillus cereus* (fig 1).It was found that *Bacillus cereus* could tolerate silver upto 800ppm which was added to the media in form of silver nitrate.

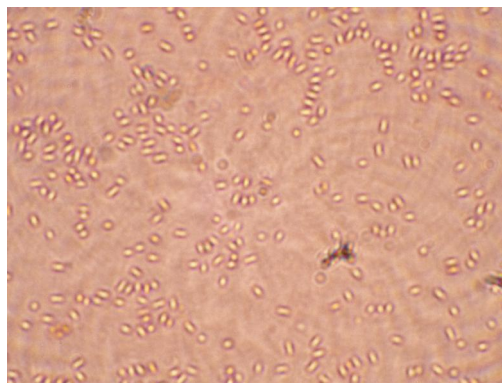


Fig 1 showing isolates of *Bacillus cereus*

Reduction was obtained in all three sets viz, when silver nitrate was added to the stationary phase , to the culture suspended in water and to the cell free suspension .recovery of silver was found to be maximum and easier in the first set hence this protocol was followed for further synthesis.Exposure to sunlight in all the three media was followed by colour change .Increase in room temperature was associated with reduction in time for the synthesis of nanoparticles. The absorbance scan of silver nanoparticles exhibited a sharp plasmon peak at 450 nm, which is characteristic of monodispersed Ag .

These particles were found to be stable in the solution on storage up to one month with no change in the absorbance maxima .The magnitude , peak wave length and spectral band width of the plasmon resonance associated with nanoparticle are dependent on particle size , shape and material composition as well as the local environment.

X-ray powder diffractogram of the biosynthesized nanosilver exhibits Bragg's reflection due to (111), (200),(220)(311)and (222) corresponding FCC type bulk silver .(fig2).

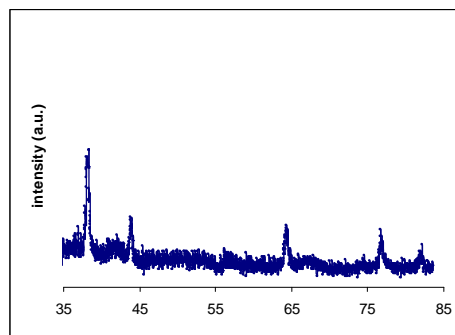


Fig. 2. XRD pattern of Silver nanoparticles

The diffraction peaks are broad around the their bases indicating that the silver particles are in nanosizes. The TEM pictures (fig3) obtained reveal that the silver nanoparticles are in the 10 nm range.

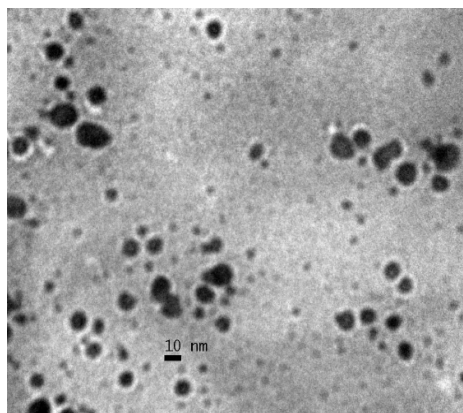


Fig. 3 Transmission Electron Microscopy  
Micrograph of silver nanoparticles  
synthesized by *Bacillus cresus*

Particles smaller than these could not be resolved. Aggregates of  $0.25\text{--}05\mu$  were observed with SEM image, probably indicating that the particles are aggregating to form larger particles. It was observed from EDAX measurements that the sample contained about 75% Ag. Though the exact mechanism is still obscure it is probable that certain enzymes are released which have reducing action. It is known that silver cations are highly

reactive and tend to bind with electron donor groups containing sulphur, nitrogen or oxygen of the proteins. Thus it is seen that the microbial biodiversity may provide cheap viable and green ways for the production of nanoparticles each unique as environment it comes from.

## CONCLUSION

In summary, a brief overview of the use of micro-organisms in the biosynthesis of Ag nanoparticles has been described. In the context of the present drive to develop green technologies in materials' synthesis, this method is a viable alternative to the physical and chemical methods currently in vogue. Extracellular secretion of enzymes offers the advantage of obtaining large quantities in a relatively pure state, free from other contaminating proteins associated with the organism. The process can be extended to the synthesis of nanoparticles of different chemical compositions, shapes and sizes by suitable identification of the enzymes secreted by the micro-organisms.

## ACKNOWLEDGEMENT

The authors wish to thank University Grants Commission, Govt. of India for providing financial support under major research project scheme

## REFERENCES

- [1] A.Krolikowska, A Kudlski., A Michota, J. Bukowska (2003) SERS studies on Structure of thioglycolic acid monolays on silver and gold. *Surf Sci.*532:227-232.
- [2] A Kumar. S Mandal, P.R Selvakanan, R Parischa, A B Mandale, M Sastry (2003) investigations into interaction between surface bound Alkylamines and gold nanoparticles .*Langmuir* 19:6277-6282.
- [3] N Chardrasekharan , PV Kamat (2000)- Improving photochemical performance by nanostructured TiO<sub>2</sub> films by absorption of gold particles .*J.Phys. Chem*104:10851-10857.
- [4] G Peto, G L.Paszi Z Molnar, Geszti , A Beck, L Guzzi,(2000). Electronic structure of gold nanoparticles deposited in SiO<sub>2</sub> /Si100.*Mater Sci.Eng* 19:95-99.
- [5] G Southam and T.J Beveridge *Geochim .Cosmochim* Acta (1996 )60, 4369-4379.
- [6] T.J Beveridge and R.G.E Murray,. (1980) *J.Bacteriol* 180 141, 867-887
- [7]. D Fortin and T.J Beveridge (2000) in *Bio-mineralisation –From Biol to Biotech &Medical applications* (ed Baeuerien E.) Wiley VCH Weinghiem p700.
- [8] E.O Wilson eds (1988) *Biodiversity* . Washington D.C natl. Acad .10.Bull A.T,
- [9] H Goodfellow & J.H Slater (1992) *Annu Review of Microb.*46:219-52.
- [10] S. Silver (1996 ) *Bacterial resistance to toxic metal ions – Review Gene* 179:9-19
- [11] Beveridge T,J. , Hughes M.N ,Lee H,leung K.T., Poole R.K. , Savvaidis I, Silver S, Trevor JT (1977) *Metal microbe interaction :contemporary approaches .Adv, Microb. Physiol.* 38:178-243.
- [12.] T Klaus, R Joerger. E Ollson, C-G Granqvist (1999) Silver based crystalline nanoparticles , microbially fabricated .*Proc. natl. Acad Sci USA* 96:13611-13614
- [13]. A Ahmad, P Mukherjee, D Mandal, S.Senapati, MI Khan ,R Kumar, M Sastry (2002) Enzyme mediated extracellular synthesis of CDS nanoparticles by the fungus *Fusarium oxysporium* .*J. Am Chem Soc.* 124:12108-12109
- [14] A Ahmad, MI Khan , S Senapati, R Kumar, M Sastry, MI Khan, (2003a) of monodisperse gold by anovel extremophilic actinomycete *Thermonospora* sp. *Langmuir*19:3550-3553
- [15]. A Ahmad, P Mukherjee , S Senapati, D. Mandal, M.I Khan ,Kumar R, M Sastry (2003c) Extracellular synthesis of silver nanoparticles using the fungus *Fusarium oxysporum* *Coll. Surf B*:313-318
- [16] D P Cunningham , LL Lundie (1993) Precipitation of cadmium y *Clostridium thermoaceticum*.*Appl. Environ. Microbiol* 59:7-14 .
- [17] D Mandal., M.E Bolander, D Mukhopadya, G. Sarkar and P Mukherjee.(2006) The use of microorganisms for the formation of metal nanoparticles and their applications .*Appl. Microbiol. Biotechnol.* 69:485-492