Available online at: http://www.ijmtst.com/vol6issue06.html

International Journal for Modern Trends in Science and Technology

ISSN: 2455-3778 :: Volume: 06, Issue No: 06, June 2020

Biosynthesis of Silver Nanoparticles through Biomass of Fungus *Aspergillus niger* and their Antibacterial Potential

Pushpendra Pratap Singh[1] | Sarika Chaturvedi[2]

[1]Research Scholar, Amity Institute of Biotechnology, Amity University, Gurugram, Haryana (India)
[2]Assistant Professor, Amity Institute of Biotechnology, Amity University, Gurugram, Haryana (India)

To Cite this Article

Pushpendra Pratap Singh and Sarika Chaturvedi, "Biosynthesis of Silver Nanoparticles through Biomass of Fungus *Aspergillus niger* and their Antibacterial Potential", *International Journal for Modern Trends in Science and Technology*, Vol. 06, Issue 06, June 2020, pp.:37-42; https://doi.org/10.46501/IJMTST060609

Article Info

Received on 28-April-2020, Revised on 20-May-2020, Accepted on 25-May-2020, Published on 03-June-2020.

ABSTRACT

Extracellular biosynthesis of silver nanoparticles by fungus Aspergillus niger which was isolated from waste water is being reported in the present paper. The production of silver nanoparticles was evidenced by UV-Vis spectrum, showing the absorbance between 260 to 400 nm. The nanoparticles characterized by Scanning Electron Microscopy exhibited silver nanoparticles with diameter of 25nm to 75nm. Energy Dispersive X-ray analysis reveals strong signals in the silver region and confirms the formation of the silver nanoparticles. The Fourier Transform Infrared Spectroscopy study confirmed that the A. niger mycomass has the ability to perform both reduction and capping functions on the silver nanoparticles. Compound Microscopy confirms the self-assembling property of silver nanoparticles. The silver nanoparticles showed remarkable antibacterial activity against Lactobacillus plantarum, Lactobacillus delbrueckii and Bacillus subtilis bacterial strains. Reduction of silver ions is an extracellular and rapid process; this information may lead to the development of easy protocols for biosynthesis of the silver nanoparticles. Antibacterial activity of silver nanoparticles is important for the development of effective antibacterial agent against those bacteria who are showing resistance against antibiotic drugs which are available in market.

KEYWORDS: *A. niger, Silver nanoparticles, Antibacterial, Biological Method.*

DOI: https://doi.org/10.46501/IJMTST060609

I. INTRODUCTION

From recent years, nanotechnology playing a very important role in the science and technology, when the significance of nanoparticles was discovered, their application also increased. Nanoparticles have unique electrical, optical and biological properties, these properties makes it applicable in catalysis, agriculture, bio sensing, imaging, drug delivery, nano device fabrication and in medicine [1]. In the past few years, there has been an increasing interest in silver nanoparticles (SNP's) on account of the antimicrobial properties that they exhibit [2]. Silver nanoparticles are projected as future generation antimicrobial agents [3].

Several physical and chemical methods have been adapted for the synthesis of metal nanoparticles. Chemical based methods are the most popular among all methods because of the fast and controlled production of nanoparticles but chemical method is not an ecofriendly approach because it can creates environmental pollution through residuals chemicals. A new approach which is currently in trend that is biological methods, these methods are ecofriendly and less expensive among all methods. Biological methods used to synthesized silver nanoparticles without the involvement of any toxic chemical [4],[5],[6],[7]. Metal ions can be reduced by the combination of different biomolecules like proteins, saccharides, vitamins etc., which can be extracted from certain organism. This process seems chemically complex but it is ecofriendly [8].

Recent studies are clear evidence of successful biosynthesis of silver nanoparticles through microbes and other biological systems [9],[10],[11]. Silver nanoparticles can be synthesized by several microorganisms such as the bacterial strains *Bacillus licheniformis*, *Klebsiella pneumonia* etc. and fungi strains such as *Verticillium*, *Fusarium oxysporum*, *Aspergillus flavus* etc. [12],[13],[14],[15],[16],[17]. Fungi secrets much amount of proteins which are catalyzed the process of formation of nanoparticles. In the process of synthesis of silver nanoparticles, firstly Ag ions traps on the surface of fungal cells than reduction of silver ions by enzymes present in fungal cell system were performed.

In the present study, silver nanoparticles were synthesized through the dead biomass of fungus *Aspergillus niger*, silver nitrate was used as a substrate material. After characterization of nanoparticles through sophisticated analytical techniques, the antimicrobial analysis was performed through agar well diffusion method with three different bacterial strains.

II. Methodology

Isolation and identification of fungus

10 ml of waste water was collected from drains near Ajanta colony, Meerut City (U.P.). Sterilized Potato Dextrose Agar medium (Raper and Thom, 1949) was used for the isolation of fungus from liquid sample. 1 ml of diluted waste water from a given sample was transferred into sterilized petri dishes followed by the addition of 20 ml cooled and sterilized PDA medium with 30 ppm of streptomycin. The Petri dishes containing medium and the inoculum were rotated (to facilitate through mixing of broth) and incubated at 26° C for 6 – 8 days. The fungal species was identified morphologically through following - Domsch and Gams (1972), Barnett and Hunter (1972), Gillman (1957), Ellish (1971, 1976), Shubramanian (1971) and Nagamani *et al.* (2006). The isolated culture of *Aspergillus niger* van teigham was grown in 500 ml Erlenmeyer flasks, each containing 100 ml PDA medium at 25–28° C under shaking condition (200rpm) for 96 hours. After 96 hours of fermentation, mycelia were separated from the culture broth by centrifugation (5000 rpm) at 10° C for 20 minutes and the settled mycelia were washed thrice with sterile distilled water to remove any medium component from the fungus biomass pellet.

Synthesis of silver nanoparticles

2 mM aqueous solution of silver nitrate ($AgNO_3$) was prepared for the synthesis of silver nanoparticles. 5 gm mycomass was taken and suspended in 100 ml of the 2 mM aqueous $AgNO_3$ solution in 250 ml Erlenmeyer flasks (at pH 5.5 - 6.0) for reduction of silver nitrate in to Ag ions. Two controls were taken, one with mycomass and distilled water (in place of $AgNO_3$ solution) and another with only 100 ml $AgNO_3$ solution. All three flasks were placed on a rotatory shaker at 28° C (at 200 rpm) and the reaction carried out for a period of 120 hours. The bio-transformation was routinely monitored visually after time periods.

Characterization

The silver nanoparticles were characterized by UV-*Vis* double beam spectrophotometer (UV- 1800 shimadzu) at various time intervals (96 hours and 120 hours). Spectra's were measured in a quartz cell at room temperature. The scanning range for the samples was 200–800 nm and scanning speed was 480 nm/min. The spectra obtained from UV-*Vis* spectrophotometer was analyzed by Gaussian function.

Scanning Electron Microscopy was carried out to characterize the size and morphology of nanoparticles. For scanning electron microscopic analysis, the dry sample was submitted to JNU, New Delhi. The film for SEM allowed to gold coating, the SEM images of these were recorded. Energy Dispersive X-ray analysis was also performed along with the SEM analysis to know about the presence of different ions and their amount in the sample.

The dry sample of silver nanoparticles and two controls, prepared by drop deposition method on a

glass slide, after completion of drying of the sample it was scratched and collected 5 mg dry sample for FTIR analysis, submitted to CCS University, Meerut.

To know about self-aggregation of silver nanoparticles, optical microscopic analysis was performed. The silver nanoparticles solution of the test biomass was deposited on a glass slide by drop deposition method and heated it on a hot plate for a while to obtain a thin film. The thin film of silver nanoparticles was observed in optical microscope (Olympus, Germany).

Antimicrobial Assay

Three bacterial strains (*Bacillus subtilis, Lactobacillus delbrueckii, Lactobacillus plantarum*) were cultured on Nutrient Agar Medium for analysis of antimicrobial potential of silver nanoparticles. The Agar Well Diffusion method applied for antimicrobial analysis of silver nanoparticles. 1 ml of bacterial sample poured in each sterilized petri plates than allowed pouring of sterilized medium and leaved the plates for solidification. 3 wells were create in each cultured plate, 1 for control (water) and 2 for sample (silver nanoparticles solution). After incubation for 24 hours at room temperature to measure the zone of inhibition.

III. Results and Discussion

Changes in the color of aqueous silver nitrate solution (exposed with mycomass) after different intervals (0, 4, 24, 48, 72, 96 and 120 hours). Lite brown color of the solution was visible after 24 hours and this color begin to darken up to 48 hours. After this period the solution shows the tendency towards transparency but simultaneously, the mycelium also started changing color and by 24 hours it assume earthen (light brown). Thereafter, the mycelium gradually became darken brown in color and started aggregating so as to far a large clump by 120 hours. The appearance of dark brown color of the fungal biomass after exposure silver ions is the clear indicators of the reduction of the silver ions and formation of silver nanoparticles. SNPs produced brown solution in water, due to the Surface Plasmon Resonances (SPR) effect [7].

UV-*Vis* spectral analysis

UV-*Vis* spectra of *Aspergillus niger* biomass, before and after emersion in the silver nitrate solution for the 120 hours, were recorded. As in the evident (Fig.1) a sharp peak was observed between 280 nm to 400 nm. As increase in the height and sharpening in the peak indicate synthesis of the nanoparticles [18]. The shifting of the peak occurs due to attachment of more and more with increasing numbers with silver nanoparticles.

A broad Surface Plasmon Resonance band is due to aggregation or adsorption of biomass onto the surface of silver nano-crystals. The UV-*Vis* absorption peak was found to fit into a Gaussian curve with all the test concentration of fungal biomass. Peak broadening was noticeable with increasing in absorbance intensity. The peak broadening is attributed to an increase in polydispersity as a result of increased biomass employed during synthesis.

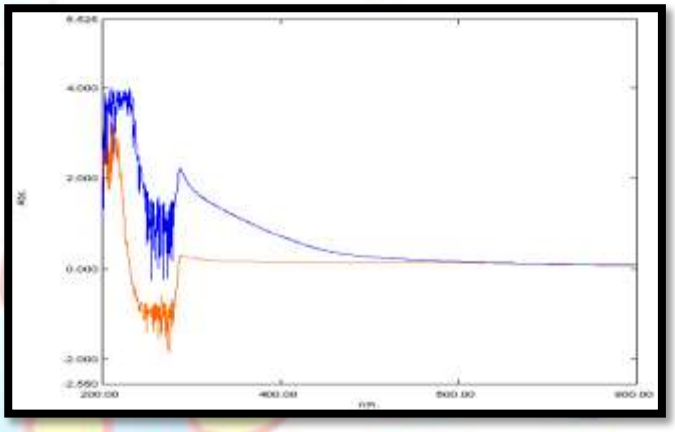

Fig.1 UV-*Vis* spectra of *A. niger* treated with silver nitrate solution (after 72 and 120 hours).

SEM and EDX analysis

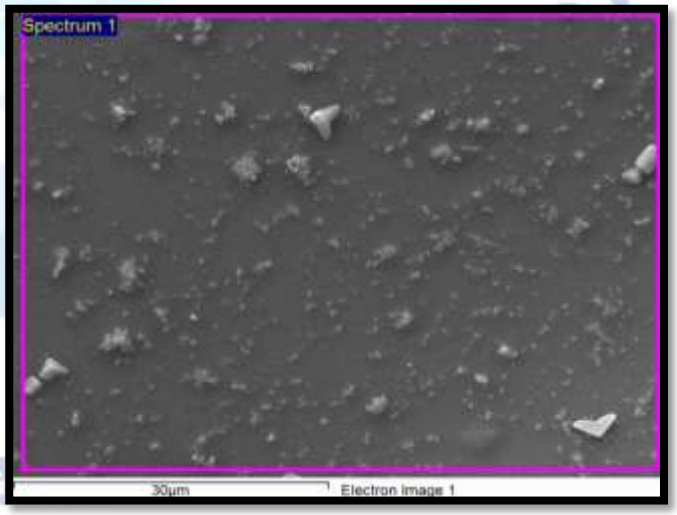

Fig.2 SEM image of biomass of *A. niger*, shows the presence of silver nanoparticles

The SEM micrograph showing the silver nanoparticles of varying sizes (Fig.2). After 120 hours of reaction of mycomass with the silver ions, the reduction and stabilization process was completed and clear mono dispersed irregular

shaped nanoparticles were observed in the SEM micrograph (25nm to 70nm).Energy Dispersive X-ray (EDX) analysis performed on a dried film of fungus extract prepared nanoparticles shows the presence of the silver (Weight 2.20% and atomic % is 0.31) in the solution (Fig.3).

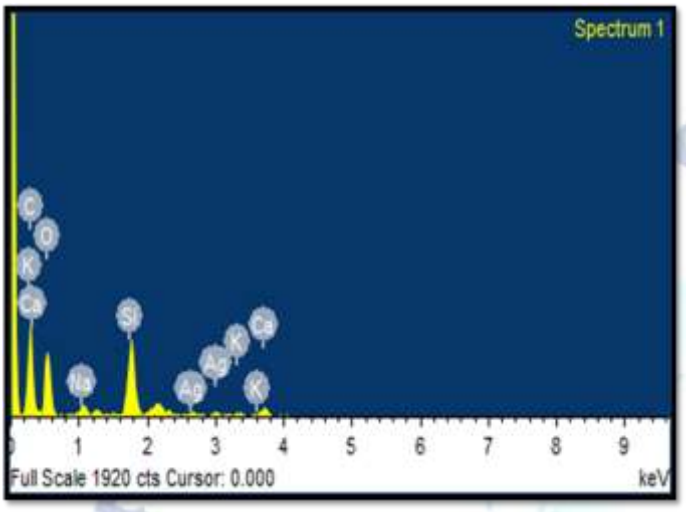

Fig.3 EDX result showing the presence of silver ions in the sample

FTIR analysis

The extract of *A. niger* mycomass with the silver nanoparticles was used for FTIR analysis. FTIR spectroscopy was used to identify the biomolecules that are present on the surface of silver and capping agent on the silver nanoparticles. The peaks of different intense were observed in micrograph of FTIR, which were analyzed to demonstrate the ability of *A. niger* mycomass to perform both reduction and capping functions on silver nanoparticles (Fig. 4).

The total number of functional groups found in silver salt (control) is 18 in numbers, similarly 13 functional groups found in FTIR analysis of dry mass of *A. niger* and 18 functional groups found in after treatment of mycomass with $AgNO_3$. In compare between $AgNO_3$, untreated dry mycomass of *A. niger* and treated sample of mycomass with silver salt, it was observed that C—C aliphatic chains, Carboxylic acid, Sulfonic acid, Alkane (—C—H), Aromatic (C=C), Aliphatic ester, C=O, P—H, Aldehyde, C=C asymmetric stretch, C≡C asymmetric stretch were disappeared in the sample (after treatment of mycomass with $AgNO_3$). It was observed that these functional groups are responsible for the attachment of silver salt to the mycomass of *A. niger*. The FTIR spectroscopic study confirmed that the *A. niger* mycomass has the ability to perform both reduction and capping functions on the silver nanoparticles.

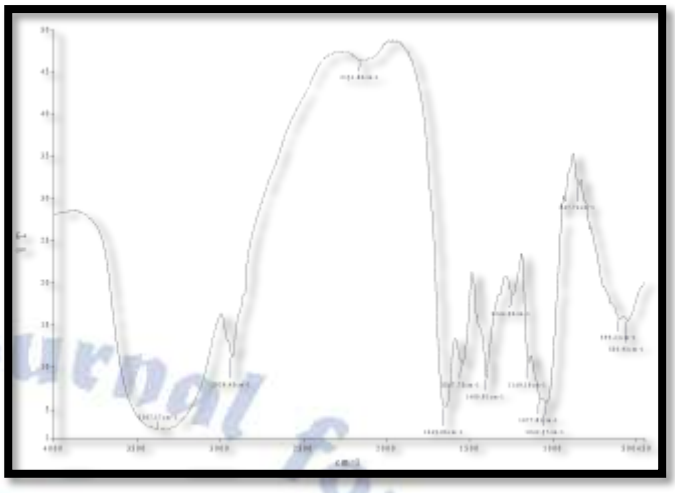

Fig.4 FTIR analysis of *A. niger* after treatment with silver nitrate

Compound microscopic analysis

When a liquid containing nanoparticles evaporates, many qualitatively different transitory structures are formed [20]. The self-assembly is governed by a suitable interplay of forces, including interactions between nanoparticles as well as capillarity and wetting-in principle, a non-equilibrium process [19]. Understanding this evolution towards the new equilibrium state at microscopic level is the first step in a systematic design of interesting and useful uniformed shape and size morphologies (Fig.5).

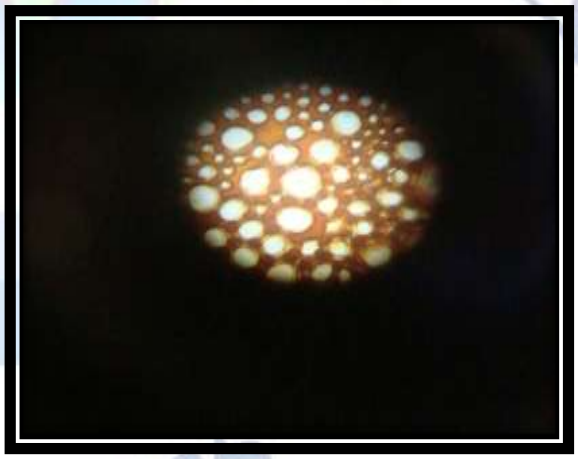

Fig.5 Image showing the self-assembled silver nanoparticles under optical microscope

Antibacterial potential of SNP's

It was previously demonstrated by many researchers that silver nanoparticles having antimicrobial potential against wide range of bacterial species. 3 different strains of bacterial species that are BS1- *Lactobacillus plantarum,* BS2- *Lactobacillus delbrueckii* and BS3- *Bacillus subtilis* were subjected for antimicrobial

assessment of silver nanoparticles by Agar Well Diffusion method. After 24 hours of incubation, as expected, the silver nanoparticles were clearly showing the antimicrobial activity against all 3 different bacterial strains with respective zone of inhibition 16 mm, 13mm and 14 mm (Fig. 6 and 7)

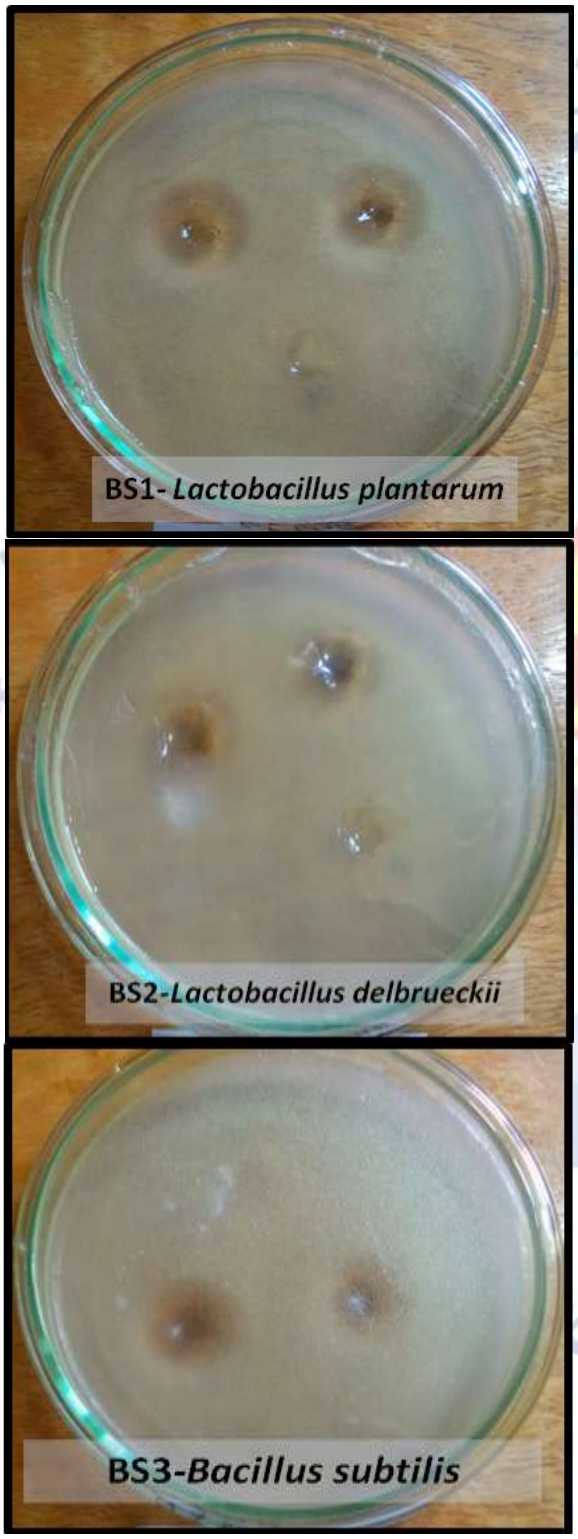

Fig.6 Antimicrobial activity of silver nanoparticles against all three strains of bacteria (BS1 to BS3) by Agar well diffusion method

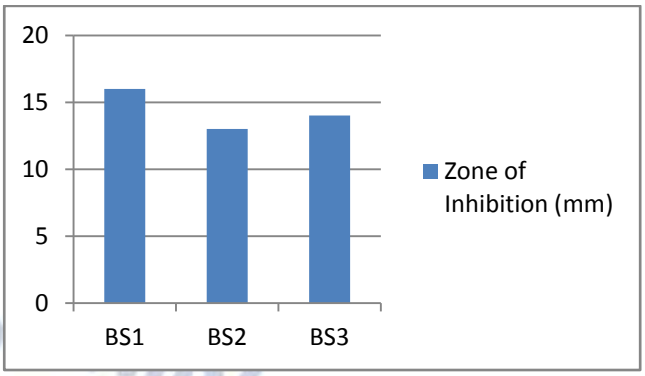

Fig.7 Zone of inhibition in three different bacterial cultures

IV. Conclusion

Simple and effective method of synthesis of silver nanoparticles with well-defined size and antimicrobial activity were performed. Silver nanoparticles have been synthesized using biomass of fungus *A. niger*. Reaction time, temperature, concentration of silver nitrate and concentration of reducing agents affect the yield and particle size of the synthesized silver nanoparticles. FTIR analysis of silver nanoparticles showed the peaks which are clearly indicating that *A. niger* has ability to perform both reducing agent and capping functions on the silver nanoparticles. The EDX analysis showed the clear sharp diffraction peak of silver ions. Compound microscopy clearly demonstrated that silver nanoparticles having self-assembling property. The antibacterial activity test examined by agar well diffusion method showed that silver nanoparticles have better antibacterial activities. We are trying to develop cost effective protocols for the synthesis of silver nanoparticles and their development as antibacterial and antifungal agent. With the analysis of nano toxicity of silver nanoparticles, we can try for the development of SNP's based antifungal and antifungal agents for social welfare.

References

[1] Nair L S and C T Laurencin, (2007). Biodegradable polymers as biomaterials. *Prog. In Polym. Sci.* 3: 6.

[2] Choi H, Perera F, Pac A, Wang L, Flak E and E Mroz E (2008). Estimating individual-level exposure to airborne polycyclic aromatic hydrocarbons throughout the gestational period based on personal, indoor and outdoor monitoring. *Environ. Health Prospect.*116: 1509–1518.

[3] Rai M, Yadav A and Gade A (2009). Silver nanoparticles as a new generation of antimicrobials. *Biotechnol. adva.* 27: 76–83.

[4] Ahmad A, Mukherjee P, Senapati S, Mandal D, Khan M I, Kumar R and Sastry M (2003a). Extracellular biosynthesis

of silver nanoparticles using the fungus *Fusarium oxysporum*. *Coll. Surf. B: Biointer*. 28: 313–318.

[5] Shankar S S, Rai A, Ankamwar B, Singh A, Ahmad A and Sastry M (2004). Biological synthesis of triangular gold nanoprisms. *Nature Mat.*3: 482–488.

[6] Ankamwar B, Damle C, Ahmad A and Sastry M (2005). Biosynthesis of gold and silver nanoparticles using *Emblica officinalis* fruit extract, their phase transfer and transmetallation in an organic solution. *J. Nanosci. Nanotechnol.*5: 1665–1671.

[7] Huang J, Li Q, Sun D, Lu Y, Su Y, Yang X, Wang H, Wang Y, Shao W, He N, Hong J and Chen C (2007). Biosynthesis of silver and gold nanoparticles by novel sundried *Cinnamomum camphora* leaf. *Nanotechnol.* 18: 105.

[8] Heinz Mehlhorn (2016). Title- Nanoparticles in the Fight against Parasites, Volume- 8 of Parasitology Research Monographs, Springer, ISBN-3319252925, 9783319252926.

[9] Korbekandi H, Iravani S and Abbasi S (2009). Production of nanoparticles using organisms. *Crit. Revi. In Biotechnol.* 29: 279–306.

[10] Sastry M, Ahmad A, Khan M I and Kumar R (2003). Biosynthesis of metal nanoparticles using fungi and actinomycete. *Curr. Sci.* 85: 162–170.

[11] Iravani S (2011). Green synthesis of metal nanoparticles using plants. *Green Chem.* 13: 2638–2650.

[12] Ahmad A, Senapati S, Khan M I, Kumar R, Ramani R, Srinivas V and Sastry M (2003). Intracellular synthesis of gold nanoparticles by a novel alkalotolerant actinomycete, *Rhodococcus* species. *Nanotechnol.* 14: 824–828

[13] Kalishwaralal K, Deepak V, Ram kumar pandian S, Bilal M and Sangiliyandi G (2008). Biosynthesis of silver nanocrystals by *Bacillus licheniformis*. *Coll. Surf. B: Biointer*. 65: 150–153.

[14] Mokhtari N, Daneshpajouh S, Seyedbagheri S, Atashdehghan R, Abdi K, Sarkar S, Minaian S, Shahverdi H R and Shahverdi A R (2009). Biological synthesis of very small silver nanoparticles by culture supernatant of *Klebsiella pneumonia*: The effects of visible-light irradiation and the liquid mixing process. *Mater. Res. Bulle.* 44: 1415–1421.

[15] Mukherjee P, Ahmad A, Mandal D, Senapati S, Sainkar S R, Khan M I, Parishcha R, Ajaykumar P V, Alam M, Kumar R and Murali Sastry (2001). Fungus-Mediated Synthesis of Silver Nanoparticles and Their Immobilization in the Mycelial Matrix: A Novel Biological Approach to Nanoparticle Synthesis, *Nano Letters*. 2001110515-519

[16] Senapati S, Mandal D, Ahmad A, Khan MI, Sastry M, Kumar R (2004) Fungus mediated synthesis of silver nanoparticles: a novel biological approach. Indian *J. Phys* 78A:101–105

[17] Vigneshwaran N, Ashtaputre N M, Varadarajan P V, Nachane R P, Paralikar K M and Balasubramanya R (2007). Biological synthesis of silver nanoparticles using the fungus *Aspergillus flavus*. *Mat. Let.* 61: 1413–1418.

[18] Sileikaite A, Puiso J, ProsycevasI, Tamulevicius S (2009). Investigation of silver nanoparticles formation kinetics during reduction of silver nitrate with sodium citrate. *Mater Sci.* 15, 21-17.

[19] Rabani E, David R R, Geissler P L and Brus L E (2003). Drying-mediated self-assembly of nanoparticles. Department of Chemistry, University of California, Berkeley, California 94720, USA.

[20] Whitesides G M, Grzybowski B, (2002). Self-assembly at all scales Science. 29; 295(5564):2418-21.