Available online at www.ijpab.com

ISSN: 2320 – 7051
Int. J. Pure App. Biosci. **2 (3):** 281-285 (2014)

Research Article

INTERNATIONAL JOURNAL OF PURE & APPLIED BIOSCIENCE

Extracellular Biological Synthesis, Characterization and Stability of Gold nanoparticles using the Fungus *Helminthosporium tetramera*

Gitanjali B. Shelar* and Ashok M. Chavan

Seed Pathology and Fungal Biotechnology Lab, Department of Botany Dr. Babasaheb Ambedkar Marathwada University, Aurangabad-431004. (M.S), India

*Corresponding Author E-mail: gitanjalibshelar@gmail.com

ABSTRACT

In the present work the fungus Helminthosporium tetramera used for the extracellular biosynthesis of gold nanoparticles. The cell filtrate of H. tetramera reacted with $AuCl^{-}4$ ions, resulting formation ofgold nanoparticles within 12 hours. The gold nanoparticles were characterized by Visual analysis, UV-Vis absorption spectroscopy and Transmission electron microscopy (TEM). The extracellular gold nanoparticles exhibited maximum absorbance at 550 nm in UV-Vis spectroscopy. UV-spectral reading clearly showed that as the incubation period increased the spectral absorbance also increased and stable after 72 hr. This stability of gold nanoparticles also constant after two month. TEM showed polydisperse spherical and occasionally triangular nanoparticles in the size range from 8-50 nm. Biological approach using the fungi is a novel way towards the safe, cost effective and ecofriendly method for the synthesis of gold nanoparticles is gaining importance in the field of nanotechnology.

Keywords: *Helminthosporium tetramera, gold nanoparticles, UV-Vis absorption spectroscopy, Transmission electron microscopy (TEM).*

INTRODUCTION

Nanotechnology is the vast growing multidisciplinary field of science which entails synthesis and development of various nanomaterials. Now different types of metal nanomaterial are being produced using Copper, Zinc, Magnesium, Silver and Gold. Due to their unusual optical[1], chemical[2], photoelectrical[3]and electronic[4]properties, nanoparticles synthesis has great interest. Today there is great demand to develop ecofriendly nanoparticle synthesis process that does not use any toxic chemicals in the synthesis. The development of synthesis of metal nanoparticles is the crucial facet of nanotechnology. Nanoparticles fascinate greater consciousness due to their various applications in different fields. Nowadays due to their emanate application; synthesis of gold nanoparticles has been the focus of interest. It is known that a large number of organisms, both unicellular and multicellular are able to produce inorganic nano materials either intracellular or extracellular[5]. Microorganism like bacteria, fungi and actinomycetes are the important tool andattractive alternative for synthesis of nanoparticles[6,7.]

Gold is the most inert of all metals. Gold nanoparticles are of interest mainly due to their stability under atmospheric conditions, resistance to oxidation, and biocompatibility[8,9].Therefore, development of techniquesfor synthesis of gold nanoparticles, of well-defined size and shape, is of great challenge.Recently, the extremophilic actinomycetes Thremonospora sp. when exposed to gold ions reduced the metal ions extracellulary, formation of gold nanoparticles[10].Filamentous fungi have been also used for the formation of colloidal gold nanoparticles [11]. The extracellular synthesis of gold nanoparticles by treatment of the fungus *Fusarium oxysporum*with aqueous AuCl4 ions has been also reported[12], and the intracellular formation of gold nanoparticlesusing the fungus Verticillium sp. was also reported [13].

In this article, the fungus *Helminthosporium tetramera*was used to synthesize gold nanoparticles. The cell filtrate of this fungus was used to develop an extracellular process for the synthesis of gold nanoparticles. Gold nanoparticles were observed within12 hours after AuCl4- solution was added to the cell filtrate.

MATERIAL AND METHODS

Collection of Materials

The fungus *H. tetramera* was isolated from soil and maintained on potato dextrose agar (PDA) medium at 30Cº. The isolated fungus was identified by lacto phenol cotton blue mounting by morphological and microscopic observation. Pure culture was maintained on potato dextrose agar slants at 30Cº.

Biomass Preparation

Glucose nutrient broth medium (GNB) was used for biomass preparation of *H .tetramera*. The flask was inoculated with spores and incubated at 28ºC on a rotatory shaker (120 rpm) for 4 days. The biomass was harvested by filtration through filter paper (Whatman filter paper no-1) and then washed with distilled water to remove any components of the medium. In a 250 mL Erlenmeyer flask five g (wet weight) was brought in contact with 100 mL of double distilled water for 3 days at 30°C and agitated again at 120 rpm. The cell filtrate was obtained by filtering it through Whatman filter paper No. 1 and the cell free filtrate was collected for experiment.

Extracellular biosynthesis of Silver Nanoparticles

The 5 mL filtrate was treated with 5 mL of 1 mM HAuCl4 solution in an Erlenmeyer flask and incubated at room temperature in dark. Control containing cell-free filtrate without Chloroauric acid solution was run simultaneously as standard with the experimental flask. All experiments were done in duplicate.

Characterization of Silver Nanoparticles

UV-visible spectroscopy analysis

Change in color of the cell free filtrate incubated with Chloroauric acid solution was visually observed over a different period of time i.e. 12, 24,36, 48, 60, 72 hr. Gold ion bio-reduction was monitored by sampling of aliquots (1 mL) at different time intervals. Absorption measurements were carried out on UV-visible spectrophotometer (Cystronics UV-Vis spectrophometer 117). UV-Visible analysis of several week old samples was also carried out to check the stability of synthesized AuNPsand absorbance was measured between 350-750 nm.

Transmission electron microscope (TEM)

For TEM measurements, a drop of synthesized AuNPs was placed on the carbon coated copper grids and kept for dry. After dryness of sample grid loaded on to a specimen holder. TEM micrographs of the sample were taken using the Morgagni 268D TEM instrument (AIIMS, New Delhi).

RESULTS AND DISCUSSION

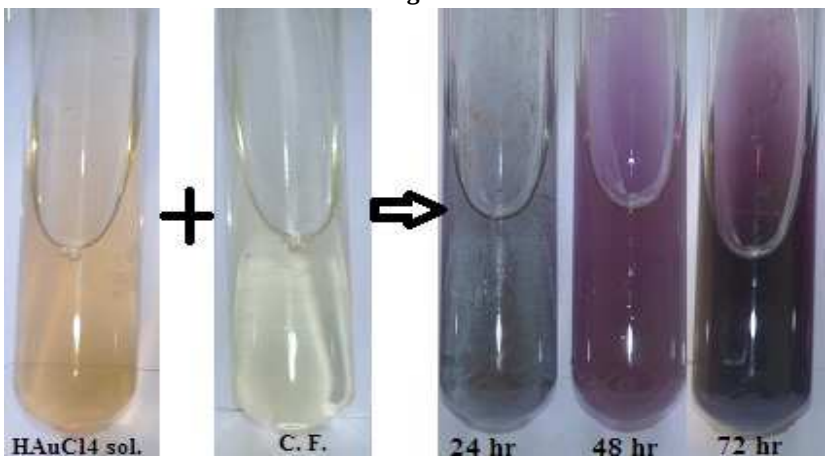
Visual Analysis

It is the preliminary test of appearance of purple color solution, after the addition of Chloroauric solution. In Figure-1 A) First test tube shows clearly golden yellow color Chloroauric acid, second test tube shows pale yellow color of cell free extract of *H .tetramera*before immersion in HAuCl4 and B) Third, fourth and fifth test tube shows different shade of purple color changes periodically after the exposure to 1 mM aqueous solution of HAuCl4 for 72 hours. It is observe that the color of gold nanoparticles periodically change from pale yellow to different shade of purple. It clearly indicates the synthesis of gold nanoparticles.

UV Spectrophotometer Analysis

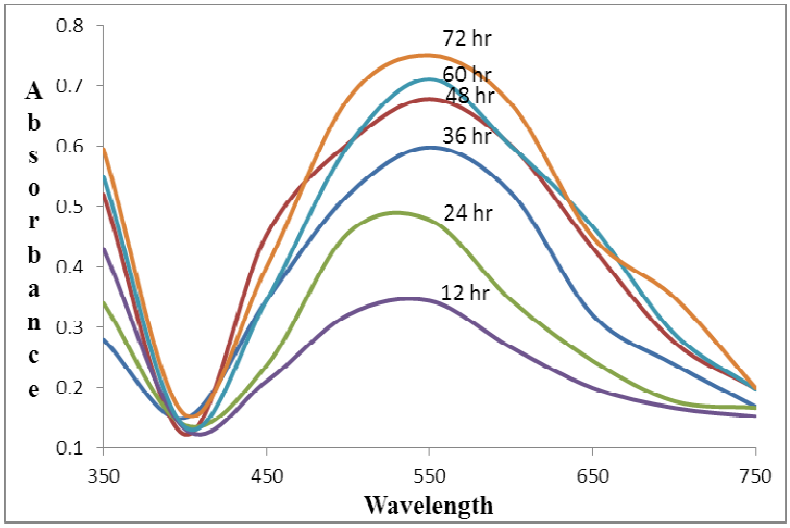
Synthesis of gold nanoparticles was monitored by UV-visible spectroscopic analysis. The UV-visible spectra of fungal cell filtrate of *H .tetramera* treated with the Chloroauric acid solution showed a characteristic surface plasmon absorption band at 550 nm, and the maximum color intensity was obtained after three days (72 hr). After 12 hours incubation UV-Visible spectral reading was lesser than other fix time interval, which clearly showed in fig 2 same type of result was observed in silver nanoparticles by Vahabi et al[14]. Beyond three days of incubation, no further increase in intensity was recorded indicating complete reduction of gold ions by the fungal cell filtrate. Synthesized AuNPs was extremely stable at room temperature, without agglomeration after 60 days was monitored regularly by UV-visible spectrophotometer. This indicated that the nanoparticles were well dispersed in the solution without aggregation, which indicates the formation of gold nanoparticles and they are stable after two months (Fig 3)

Fig-1



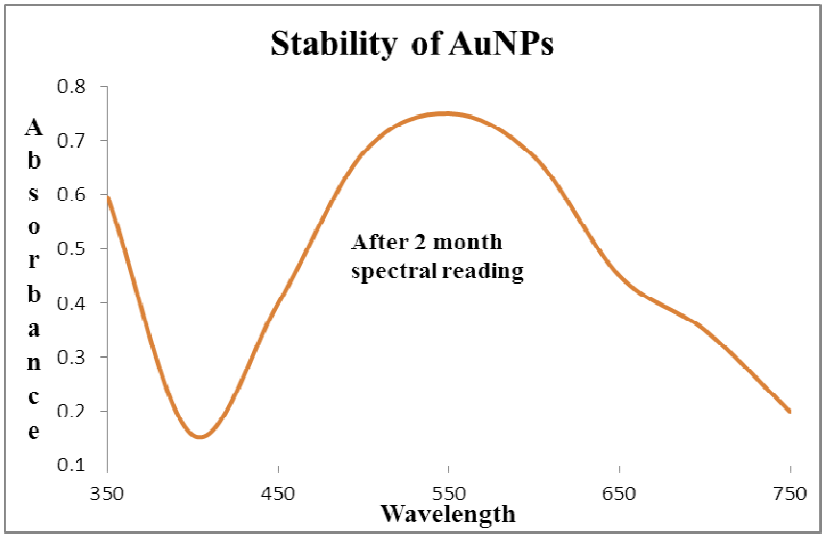
A) First test tube shows the 1 mM Chloroauric acid solution and second test tube shows fungal cell free extract of *H .tetramera* and (B)Shows the periodically color changes from pale yellow to dark brown with 1mM aqueous solution of AgNo3

Fig-2



UV-visible spectra recorded peak formation from fungal cell free extract after the immersion of 1mM HAuCl4 solution after different time interval (12, 24, 36, 48, 60 and 72 hours)

Fig-3

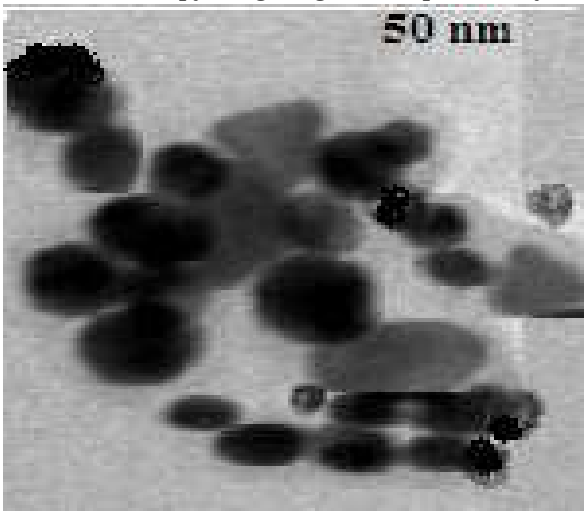


UV-visible spectra recorded peak formation from fungal cell free extract after 60 days incubation

TEM Analysis

TEM micrograph provided detailed morphology of gold nanoparticles. The data obtained from micrograph images showed distinct shape and size of polydisperse nanoparticles. Mostly particles were spherical, ellipsoidal and occasionally triangular in shape in the range of 8-50 nm with average 34.50 nm in size without significant agglomeration (Fig-3).

Fig-4: Transmission electron microscopy image of gold nanoparticles synthesized by *H. tetramera*



CONCLUSION

Nowadays there is an increasing demand to prepare AuNPs for different medical and industrial purposes. The synthesis of AuNPs using a cell free extract of *Helminthosporium tetramera* appears to be simple and an appropriate method for synthesis of AuNPs also this approach would be suitable for developing a biotechnological process for large-scale production of small AuNPs.The present study demonstrated the biosynthesis of gold nanoparticles by cell free extract of *Helminthosporium tetramera* using 1 mM Chloroauric acid. These gold nanoparticles are found to have strong absorption peak at 550 nm and stable after long time without any clumping. The TEM result shows the synthesis of polydisperse spherical and triangular gold nanoparticles of the size range 8-50 nm with no agglomeration. Results conclude that isolated *Helminthosporium tetramera* is a prominent producer of gold nanoparticles.

Acknowledgement

The authors are grateful to Anatomy department of All India Institute of Medical Sciences (AIIMS), New Delhi for providing TEM characterization facility. Authors are also thankful to Professor and HOD, Dept. of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad for giving research facilities.

REFERENCES

1. Krolikowska, A. Kudelski, A. Michota, A. Bukowska, J. *J. Surface Sci*., 532, 227 (2003)
2. Kumar, A. Mandal, S. Selvakannan, P. Parischa, R. Mandale, A. Sastry, M. Langmuir, **19**: 6277 (2003)
3. Chandrasekharan, N. and Kamat, P. *J. Phys. Chem. B*., 104, 10851 (2000)
4. Peto, G. Molnar, G. Paszti, Z. Gesztio,. Beck, A. Guczi, l., *Mater. Sci. Eng*., **19**: 95 (2002)
5. Dickson, D. P. E. and Magn, J., *J. Adv. Mater Sci*., **46**: 203 (1999)
6. Klaus-Joerger, R. Olsson, E. and Granqvist, C. G., *Proceedings of the National Academy of Sciences of the United States of America*, **96**: 24, 13611(1999)
7. Mukherjee, P. Ahmad, A. and Mandal D., Nano Letters, **1**: 10, 515 (2001)
8. Gericke, M. and Pinches, A., *Gold Bulletin*, **39**: 1-22 (2006)
9. Huang, J. Li, Q. and Sun, D., Nanotechnology, **18**: 10, Article ID 105104 (2007)
10. Sastry, M. Ahmad, A. and Pasrichaa, R., *J. Mater. Chem*., **131**: 822 (2003)

11. Sugunan, A, Melin, P. Schnurrer, J. Hilborn, J. Dutta, *J. Adv. Mater. Sci*. **321**: 4576 (2001)
12. Mukherjee, P. Senapati, S. Mandal, D. Ahmad, A. Khan, M.I. Kumar, R., Sastry, M., *J. Chem. Bio.*, **3**: 461 (2002)
13. Sujoy, K. D. and Enrico, M., *Rev. Environ. Sci. Bitechnol*., **5**: 32 (2010)
14. Vahabi, K. Mansoori, G. and Sedighe, K., *In Science Journal*, **1**: 1-65 (2011)