Australian Journal of Basic and Applied Sciences, 6(1): 86-88, 2012
ISSN 1991-8178

Instantaneous Biosynthesis of Silver Nanoparticles by Selected Macro Fungi

Yen San, Chan, Mashitah, Mat Don

School of Chemical Engineering, Universiti Sains Malaysia Engineering CampusNibong Tebal, Seberang Perai Selatan, Penang, Malaysia

Abstract: Silver nanoparticles (AgNPs) have been reported to be useful in various medical and life sciences related application. It has received considerable attention in order to develop an efficient methodology for its synthesis. Conventional chemical synthesis is said to be harmful when used in medical related applications. Hence, this study was carried out with locally isolated fungi to synthesize silver nanoparticles biologically. Three modes of biosynthesis were identified: biosynthesis with the mycelia for extracellular and intracellular cells synthesis and sans mycelia for culture free synthesis. The white rot fungus, *Schizophyllum commune*, was found to have an ability to produce AgNPs instantaneously. The biosynthesis of AgNPs was further confirmed with gas chromatography-mass spectroscopy (GC-MS), UV-visible spectroscopy, and Zetasizer Nano ZS.

Key words: silver nanoparticles, biological synthesis, bionanotechnology, *Schizophyllum commune*.

INTRODUCTION

Silver has been used since time immemorial in the form of metallic silver, and silver sulfadiazine for the treatment of wounds, burns, and several bacterial infections problem (Catauro, *et al*., 2004; Crabtree, *et al*., 2003) . The synthesis of metal nanoparticles is the current research trend because they exhibit different physical and chemical properties compared to their bulk metals (Gratzel, 2001; Xia, 2003). This is due to the large surface area obtained in the nanosize particles where the chemical properties of the metals are intensified. The current industrial trend is dealing with the production of silver nanoparticles which is highly biocompatible, cheap and environmental friendly. Therefore, biosynthesis methods have been investigated as an alternative to chemical and physical synthesis.

It was reported that the cell biosynthesis is associated with silver nanoparticles. These methods can be divided into three categories depending on the place where nanoparticles are created, i.e. intra, extracellular(Reference method for broth dilution antifungal susceptibility testing of yeast, 1997) and supernatant extract Shahverdi, *et al*., 2007). The use of eukaryotic organisms such as fungi holds a promise for large scale metal nanoparticles production as the enzymes secreted by fungi is an essential element for the biosynthesis of metal nanoparticles (Das and Marsili, 2010). Different fungi such as *Verticillium*, *Fusarium oxysporum* and *Colletotrchum sp.* have been reported to synthesize metal nanoparticles (Shankar, *et al*., 2003; Sastry, *et al*., 2003; Ahmad, *et al*., 2003; Mohammed Fayaz, *et al*., 2009; Mukherjee, *et al*., 2001; Mandal, *et al*., 2006) . However, publication on the synthesis of AgNPs by locally isolated fungi is less reported. Hence, this study was carried out to determine the biosynthesis of AgNPs by locally isolated fungi. The finding of this study is crucial especially in the medical and life sciences industry to identify the best mode of silver nanoparticles production from locally isolated fungi strains *Pycnoporus sanguineus*, *Schizophyllum commune* and *Lentinus sajar caju* before incorporating its applications in medicine and health such as drug delivery and medical diagnostics.

MATERIALS AND METHODS

A. Macro fungus:

Locally isolated macro fungus, *Pycnoporus sanguineus*, *Schizophyllum commune*,and *Lentinus sajor caju* were used in this study and were obtained from Forest Research Institute of Malaysia (FRIM), Kepong, Malaysia.

B. Preparation of mycelium and supernatants:

The tested fungi were inoculated into 250 mL Erlenmeyer flasks, each containing 50mL of semi defined medium (SDM) composed of KH_2PO_4 (7g/L), K_2HPO_4 (2g/L), $MgSO_4.7H_2O$ (0.1g/L), $(NH_4)_2SO_4$ (0.1 g/L), yeast extract (0.6g/L), and glucose (10g/L) at 30^oC under shaking condition (200 rpm) for 96 h. After 96 h of cultivation, mycelia were separated from the culture broth by centrifugation at 4500 rpm, 10^oC, for 15 min. The settled mycelia were washed thrice with deionized water.

Correspoding Author: Mashitah, Mat Don, School of Chemical Engineering, Universiti Sains Malaysia Engineering Campus Nibong Tebal, Seberang Perai Selatan, Penang, Malaysia
Tel: (604)-5946468
Fax: (604)-5941013
E-mail: chmashitah@eng.usm.my/

C. Biosynthesis of silver nanoparticles:

The 1% of washed mycelia was then inoculated into aqueous silver nitrate solution (10^{-3}M). The mixtures were thereafter incubated in a rotary shaker at 200 rpm in the dark at 30°C. The bioreduction of silver nitrate into AgNPs were monitored periodically by visual inspection and in a UV-visible spectrophotometer. The UV-vis spectra of these samples were measured using Shidmazu, UV-2550 UV-visible spectrophotometer operated at a resolution of 1nm. For intracellular identification, mycelia were re-suspended in phosphate buffer saline (pH 7.4) and homogenized using a sonicator at a frequency of 8.5 Hz for 5 min. The culture supernatants were inoculated into $AgNO_3$ and cultured using similar condition. Particle size distributions of these samples were also obtained using Zetasizer Nano ZS (Malvern Instruments, Southborough, UK). Furthermore, the bioreduction of silver by reducing agent was identified using gas chromatography-mass spectroscopy (Perkin-Elmer Clarus 600).

RESULTS AND DISCUSSIONS

A. Silver reduction:

The biological synthesis of AgNPs by different fungal strains was investigated. The appearance of a cloudy light-grey solution in the Erlenmeyer flasks indicated a reduction of silver ion and the formation of silver nanoparticles has taken place. Figure 1 shows two Erlenmeyer flasks with *Schizophyllum commune* mycelia before (left) and after (right) the reaction with $AgNO_3$. The colour changes were observed immediately when the tested fungus, *Schizophyllum commune* was transferred into the flask containing silver nitrate. However, for *Pycnoporus sanguineus*, and *Lentinus sajor caju*, the colour changes can only be observed after 1 day of incubation .

$$Ag^{+}(aq) + e^{-} \rightarrow Ag(s) \quad (1)$$

According to Maroto and co-researcher (Duran, *et al*., 2005), bioreduction indicates the presence of reducing agent which served as electron shuttle in this reduction reaction (Eq. 1), and it was also reported that, fungi mediated reduction were most probably either by the action of a reductase or by electron shuttle quinones or both (Duran, *et al*., 2005) . In fact it was shown that, the presence of hydrogenase and nitrate reductase (Duran, *et al*., 2005; Ottow and Von Klopotek, 1969) were the essential proteins for metal reduction. In this study, the reduction of silver could be due to a reduction of diketone compound as was confirmed though a GC-MS analysis shown in Figure 2.

B. Localized surface plasmon polarization characterization:

UV-visible spectroscopy can be used to track the size of nanoparticles by electron charge oscillation principle, surface plasmon polarization, in silver nanoparticles that exhibit by light Henglein, 1993; Sastry, *et al.*, 1997) . A strong, broad peak located between wavelengths 370 and 390 nm were observed. These peaks were showed the characteristic of the plasmon band for AgNPs formed at different time intervals Mulvaney, 1996) . In this study, wavelength of 380 nm was selected for further identification of optical density (OD) of AgNPs production. It was found that the OD of AgNO3 reacted with *Schizophyllum commune*, *Lentinus sajor caju* and *Pycnoporus sanguineus* were in the descending order 0.190 > 0.05 > 0.02 respectively. For intracellular cells, all the tested fungi showed instantaneous production of AgNPs, with OD of 0.733 (*S. commune*), 0.624 (*L. sajor caju*), and 0.576 (*P. sanguineus*).

C. Particle size:

Figure 3, 4 and 5 shows the particle size distribution of AgNPs produced using *Pycnoporus sanguineus*, *Schizophyllum commune,* and *Lentinus sajor caju* at 48 hours. It was found that the culture broth for *Pycnoporus sanguineus,* and *Schizophyllum commune* did not produce any silver nanoparticles whereas the culture broth of *Lentinus sajor caju* could produce nanoparticles with an average diameter of 53 nm (Figure 5). From the extracellular secretion of *Schizophyllum commune*, *Lentinus sajar caju* and *Pycnoporus sanguineus*, it was observed that the AgNPs nano-diameter sizes were 42.12 nm, 89.76 nm, and 120.6 nm, respectively. As for the intracellular secretion analysis, nano-diameter size identified were consistent at about 50-60 nm.

Conclusions:

Although there are numerous publications on biological synthesis of AgNPs using bacteria, the process is rather slow for complete reduction to produce AgNPs. The tested fungus, *Schizophyllum commune* was able to produce AgNPs instantaneously while *Pycnoporus sanguineus*, and *Lentinus sajor caju* required 2 days for bioreduction to take place, thus indicating that the process is rather fast for complete bioreduction to produce AgNPs. Also, a hypothetical mechanism of AgNPs synthesis through reduction of heptan-2,6-dione was identified.

ACKNOWLEDGMENT

This work is supported and funded by Ministry of Higher Education Malaysia through Fundamental Research Grant Scheme (FRGS/1/10/TK/USM/02/5) and USM fellowship which we gratefully acknowledge.

REFERENCES

Ahmad, A., P. Mukherjee, S. Senapati, D. Mandal, M.I. Khan, R. Kumar, and M. Sastry, 2003. "Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*," *Colloids and Surfaces B: Biointerfaces*, 28: 313-318.

Catauro, M., M.G. Raucci, F.D. De Gaetano and A. Marotta, 2004. "Antibacterial and bioactive silver-containing Na2O X CaP X2SiO2 glass prepared by sol-gel method," *Journal of Material Science: Material in Medicine*, 15: 831-837.

Crabtree, J.H., R.J. Burchette, R.A. Siddqi, I.T. Huen, L.L. Handott and A. Fisherman, 2003. "The efficacy of silver-ion implanted catheters in reducing peritoneal dialysis-related infections," *Peritoneal Dialysis International*, 23: 368-374.

Das S.K., and E. Marsili, 2010. "A green chemical approach for the synthesis of gold nanoparticles: characterization and mechanistic aspect," *Reviews in Environmental Science and Biotechnology*.

Duran, N., P.D. Marcato, O.L. Alves, D.G.I. Souze and E. Esposito, 2005. "Mechanistic aspects of biosynthesis of silver nanoparticles by Fusarium oxyporum strains," *Journal of Nanobiotechnology*, 3.

Gratzel, M., 2001. "Photoelectrochemical cells," *Nature*, 414: 338-344.

Henglein, A., 1993. "Physiochemical properties of small metal particles in solution: "micoelectrode" reaction, chemisorption, composite metal particles and the atom-to metal transition," *Journal of Physical Chemistry B*, 97: 5457-5471.

Mohammed Fayaz, A., K. Balaji, P.T. Kalaichelvan and R. Venkatesan, 2009. "Fungal based synthesis of silver nanoparticles--an effect of temperature on the size of particles," *Colloids Surf B Biointerfaces*, 74: 123-126.

Mukherjee, P., A. Ahmad, D. Mandal, S. Senapati, S.R. Siainkar, M.I. Khan, R. Parischa, P.V. Ajyakumar, M. Alam, R. Kumar, and M. Sastry, 2001. "Fungus mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: A novel biological appraoch to nanoparticle synthesis," *Nano Letters*, 1: 515-519.

Mandal, D., M.E. Molander, D. Mukhopadhyay, G. Sarkar and P. Mukherjee, 2006. "The use of microorganism for the formation of metal nanoparticles and their application," *Applied Microbiology and Biotechnology*, 69: 485-492.

Mulvaney, P., 1996. "Surface Plasmon Spectroscopy of Nanosized Metal Particles," *Langmuir*, 12: 788-800.

Ottow, J.C.G. and A. Von Klopotek, 1969. "Enzymatic reduction of iron oxide by fungi," *Applied Microbiology* 27: 411-425.

"Reference method for broth dilution antifungal susceptibility testing of yeast; third informational supplement, 1997. " vol. Approved standard M27-A, ed: Wayne (Penn):CLSO.

Shahverdi, A.R., S. Minaeian, H.R. Shahverdi, H. Jamalifar and A. Nohi, 2007. "Rapid synthesis of silver nanoparticles using culture supernatants of Enterobacteria: A novel biological approach," *Process Biochemistry*, vol. 42, pp. 919-923.

Shankar, S.S., A. Ahmad, R. Parischa, and M. Sastry, 2003. "Bioreduction of chloroaurate ions by geranium leaves and its endophytic fungus yields gold nanoparticles of different shapes," *Journal of Material Chemistry*, 13: 1822-1826.

Sastry, M., A. Ahmad, M.I. Khan and R. Kumar, 2003. "Biosynthesis of metal nanoparticles using fungi and actinomycete," *Current Science*, 85: 162-170.

Sastry, M., V. Patil and S.R. Sainkar, 1997. "pH dependent changes in the optical properties of carboxylic acid derivatized silver colloidal particles," *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 127: 221-228.

Xia, Y., P. Yang, Y. Sun, Y. Wu, B. Mayers, Y. Gates, Y. Yin, and H. Yan, 2003. "One-dimensional nanostructures: synthesis, characterization and applications," *Advanced Materials*, 15: 353-389.