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Instantaneous Biosynthesis of Silver Nanoparticles by Selected Macro Fungi

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Abstract: Silver nanoparticles (AgNPs) have been reported to be useful in various medical and life sciences related application. Therefore, the synthesis of AgNPs has received considerable attention in order to develop efficient methodology for its synthesis. Conventional chemical synthesis is said to be harmful when used in medical related applications. Hence, current research involving biological synthesis of AgNPs is performed. Several locally isolated fungi were screened and we discovered silver nanoparticles (AgNPs) were produced after treating silver nitrate with fungus mycelia/culture supernatant. Three modes of biosynthesis were identified: with the mycelia, extracellular and intracellular biosynthesis is possible and thirdly though the culture supernatant sans mycelia. From this research, we also confirmed that *Schizophyllum commune* has the ability to produce AgNPs instantaneously. The biosynthesis of AgNPs is further confirmed with gas chromatography-mass spectroscopy (GC-MS), UV-visible spectroscopy, and Zetasizer Nano ZS.

Key words: silver nanoparticles, biological synthesis, bionanotechnology.

INTRODUCTION

Silver has been used since time immemorial in the form of metallic silver, and silver sulfadiazine for the treatment of wounds, burn, and several bacterial infections problem (Catauro, M., 2004; Crabtree, J.H., 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, M., 2001; Xia, Y., 2003). This is due to the large surface area obtained in the nanoparticles 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 (Brewer, M., 2007) and supernatant extract (Shahverdi, A.R., 2007). The use of eukaryotic organisms such as fungi holds promise for large scale metal nanoparticles production as the enzymes secreted by fungi is an essential element for the biosynthesis of metal nanoparticles (Das, S.K. and E. Marsili, 2010). Different fungi such as *Verticillium*, *Fusarium oxysporum* and *Colletotrchum sp.* have been reported to synthesize metal nanoparticles (Shankar, S.S., 2003; Sastry, M., 2003; Ahmad, A., 2003; Mohammed A. Fayaz, 2009; Mukherjee, P., 2001; Mandal, D., 2006). However, less publication on the synthesis of AgNPs by locally isolated fungi is reported. Hence, this study was carried out to determine the biosynthesis of AgNPs by locally isolated fungus. The finding of this study is crucial especially in the medical and life sciences industry to identify the silver nanoparticles production mode of locally isolated fungus strain *Pycnoporus sanguineus, Schizophyllum commune* and *Lentinus sajar caju* before incorporate into medical applications such as drug delivery and medical diagnostics.

MATERIAL AND METHODS

A. Macro Fungus:

Locally isolated macro fungus, *Pycnoporus sanguineus, Schizophyllum commune,*and *Lentinus sajor caju* are used in this study and are 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.

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C. *Biosynthesis of Silver Nanoparticles:*

The 1% of washed mycelia were 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 theses 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

D. *Silver Reduction:*

The biological synthesis of AgNPs by different fungi strains was investigated. The appearance of cloudy light-grey colour in the Erlenmeyer flask indicated a reduction of silver ion and the formation of silver nanoparticles has taken place. Figure 1 shows two Erlenmeyer flask with *Schizophyllum commune* mycelium before (left) and after (right) the reaction with $AgNO_3$. The colour changes were observed immediately when the tested fungus 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 the incubation period.

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

According to Maroto and co-researcher, bioreduction indicates the presences of reducing agent which served as electron shuttle in this reduction reaction and it was also reported that, fungus reduction was most probably either by reductase action or by electron shuttle quinones or both (Duran, N., 2005). In fact it was shown that, the presence of hydrogenanse and nitrate reductase (Duran, N., 2005; Ottow, J.C.G. and A. Von Klopotek, 1969) are the essential element for metal reduction. In this study, the reduction of silver could be due to a the reduction of diketone compound as was confirmed though a GC-MS analysis shown in Figure 2.

Fig. 1: Solution of silver nitrate before(left) and after (right) exposure to the culture of *Schizophyllum commune.*

E. *Localized Surface Plasmon Polarization Characterization:*

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

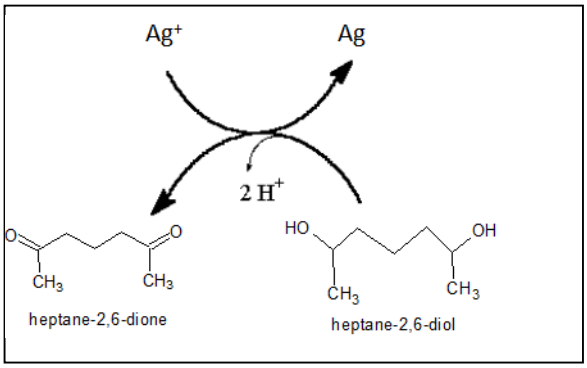

Fig. 2: Hypothetical mechanism of silver nanoparticles biosynthesis analysed using GC-MS Perkin-Elmer Clarus 600.

F. ***Particle Size:***

Figure 3, 4 and 5 showed the particle size distribution of AgNPs produced using *Pycnoporus sanguineus*, *Schizophyllum commune*, and *Lentinus sajor caju* at 48 hour. It is observed that culture broth for *Pycnoporus sanguineus*, and *Schizophyllum commune* did not produced any silver nanoparticles whereas culture broth of *Lentinus sajor caju* could produce nanoparticles with average diameter of 53 nm (Figure 5). From the extracellular secretion of *Schizophyllum commune*, *Lentinus sajar caju* and *Pycnoporus sanguineus*, it is observed that 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.

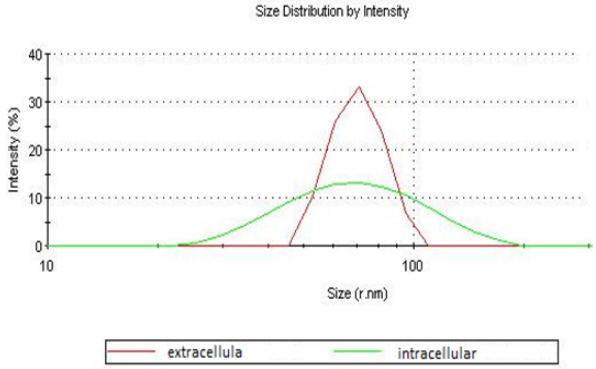

Fig. 3: Particles size distribution for AgNPs produced using *Pycnoporus sanguineus* at 48h.

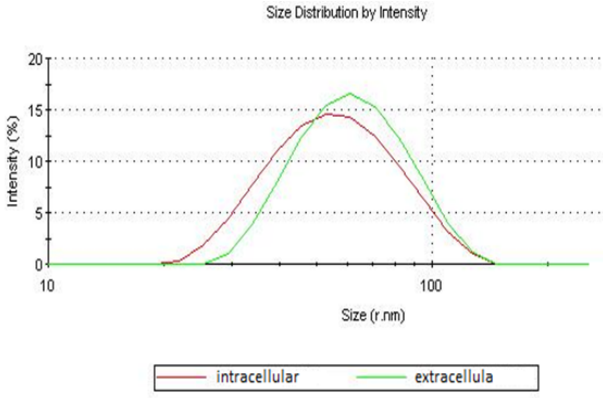

Fig. 4: Particles size distribution histogram for AgNPs produced using *Schizohyllym commune* at 48h.

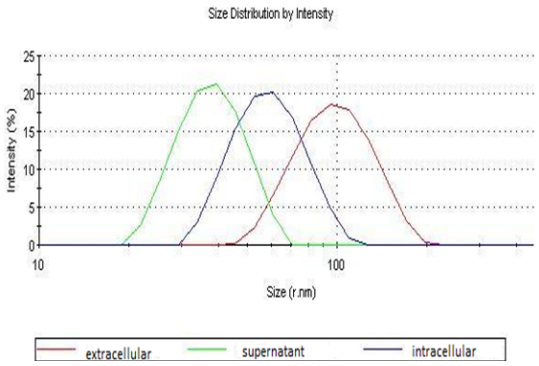

Fig. 5: Particles size distribution histogram for AgNPs produced using *Lentinus sajor caju* at 48h.

Conclusions:

Although there are lots of publications on biological synthesis of AgNPs using bacteria or fungi, the process is rather slow for complete reduction to produce AgNPs. The tested fungus, *Schizophyllum commune* was able to produce 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.

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