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Green Synthesis of Gold Nanopartcles using *Candida cylindracea*

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The green synthesis of nanoparticles that have environmentally acceptable solvent systems and eco-friendly reducing agents is of great importance. Meanwhile, the synthesis of metal nanoparticles of different sizes, shapes, chemical composition and controlled monodispersity is a key area of research in nanotechnology because of their fascinating physical properties and technological applications. As such, biosynthesis of gold nanoparticles with small size and biostability is vital and used in various biomedical applications. In this paper we report the extracellular biosynthesis of gold nanoparticles (AuNPs) using *Candida cylindracea* (also known as *Candida rugosa*), yeast. In the biosynthesis of AuNPs using this yeast, the cultured yeast was exposed to the chloroaurictrihydrate. The bioreduction process involves Au^{+} ions which are reduced to metallic AuNPs through the catalytic effect of the extracellular enzyme. These gold nanoparticles were characterized by the means of UV–Vis spectroscopy,transmission electron microscopy (TEM), X-ray Diffraction spectrum (XRD) and Fourier transform infrared spectroscopy (FTIR), UV–visible spectrum of the aqueous medium containing auric ion showed a peak at 530 nm corresponding to the surface plasmonresonance of gold nanoparticles. The intensity of the colour was found to increase with respect of time. TEM micrograph showed the formation of well-dispersed gold nanoparticles in the range of 10–30 nm with spherical and triangular shape. Fourier transform infrared spectroscopy revealed possible involvement of reductive groups on the surfaces of nanoparticles. Hence the present study enlightens the green chemistry approach on the production of gold nanoparticles using a microorganism. In comparison to chemical synthesis, the synthesis of gold nanoparticles by microbial source is an environmental friendly method for AuNP production.

Key word: Gold Nanoparticles, Extracellular Biosynthesis,
Surface plasmon resonance. Transmission Electron Microscope.

There is a growing need to develop environmentally benign nanoparticle synthesis processes that do not use toxic chemicals in the synthesis protocol. Even though many biotechnological applications such as remediation of toxic metals employ microorganisms such as bacteria[1] and yeast[2], such microorganisms were recently found as possible eco-friendly nanofactories[3,4]. Processes devised by nature, for the synthesis of inorganic materials on nano and microlength scales have contributed to the development of a relatively new and largely unexplored area of research based on the use of microbes in the biosynthesis of nanomaterials[5].

Gold is a rare element which has been used to treat different diseases such as cancer diagnostics and therapy. The specifications of gold nanoparticles differ from bulk gold in size and shape[6,7]. Gold nanoparticles have been used as anti-HIV, anti- angiogenesis, anti-malarial and anti-arthritic agent[7,8]. Furthermore, gold nanoparticles are used for delivering molecules into cells to slow

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down cancer cell growth and/or destroy cancer cells[9]. They play a major role in the treatment of cancer due to its biocompatibility and strong interaction with soft bases such as thiols.

The supernatant used for the synthesis of nanoparticles and the downstream processing of the supernatant is much simpler handle. The synthesis process is of low-cost and non-toxic[10]. The objective of this study is to demonstrate the feasibility of a yeast/enzyme-based *in vitro* approach for the biosynthesis of gold nanoparticles from *Candida cylindracea,*

MATERIALS AND METHODS

The yeast, *Candida cylindracea* was isolated and cultured. Culture was grown up in a conical flask containing 100 ml of nutrient broth in a shaker incubator at 25°C. After 96 h of incubation, biomass was separated from the culture supernatant by centrifuging it at 5000 rpm, 10°C for 10 minutes. Then the culture supernatant was mixed with 10^{-3}M aqueous Auric Chloride ($HAuCl_4$). Then the reaction mixture was left for a further 24-72 h in a shaker incubator at 30 °C.

Biotransformation took place after incubation i.e., chloroaurate ions were reduced to gold nanoparticles. The accumulation and reduction of gold were examined by visual observation of the medium. The medium turned from pale yellow to purple which was a clear indication of the formation of gold nanoparticles. The synthesized gold nanoparticles were characterized by UV-Visible Spectroscopy, Transmission Electron Microscopy (TEM), X-ray Diffraction analysis (XRD) and Fourier Transform Infra-Red Spectroscopy (FTIR).

RESULTS AND DISCUSSION

In this investigation, the selected yeast *Candida cylindracea* was screened and found to be successful in producing gold nanoparticles of uniform size and distribution, and quit stable in the solution. The yeast, *Candida cylindracea* incubated with broth containing auric chloride solution at 30 °C for 48 h. The pH value was selected in the range of 6-7.

The auric chloride ions were reduced during the exposure to the culture supernatant of the yeast and as a result biotransformation took place. The color of the reaction solution turned from pale yellow to purple indicating the formation of gold nanoparticles (Figure 1). Control experiments without culture supernatant addition stayed pale yellow, indicating that the production of gold nanoparticles was obtained by the reduction of bioliquids from the yeast indeed.

The formation of gold nanoparticles was monitored by UV- Visible spectroscopy. As the size of the gold nanoparticles increases, the color of the solution varied from deep red to purple (Figure 1). The different colors of gold nanoparticle solution are due to their Surface Plasmon

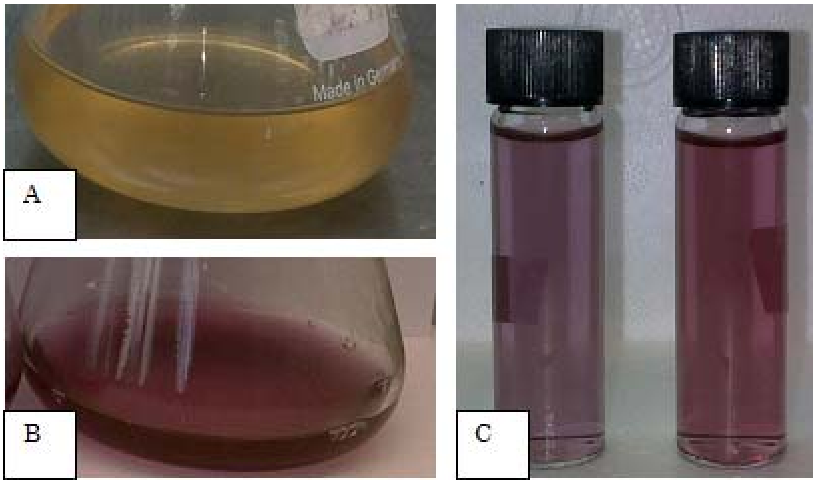

Fig. 1. Biosynthesised gold nanoparticles in a culture supernatant using *Candida cylindracea*.(A) Control sample, (B) and (C) pale yellow turned to purple

Resonance properties. Nanoparticles can experience surface plasmon resonance (SPR) in the visible portion of the electromagnetic spectrum. This means that a certain portion of a visible wavelength will be absorbed and while another portion will reflect. The portion reflected will lend the material a certain color. After 24 h of incubation, the spectroscopic studies revealed the absorption maxima of 530 nm (Figure 2).

The TEM images recorded from drop-coated films of the gold nanoparticles synthesized by treating gold chloride solution with *Candida cylindracea* are shown in Figure 3. A large density of spherical gold nanoparticles can be seen and the size of nanoparticles are from 10–30 nm. Some triangular shaped particles are also present. Interestingly, particles are quite separated; this may be due to some bioorganic component secreted

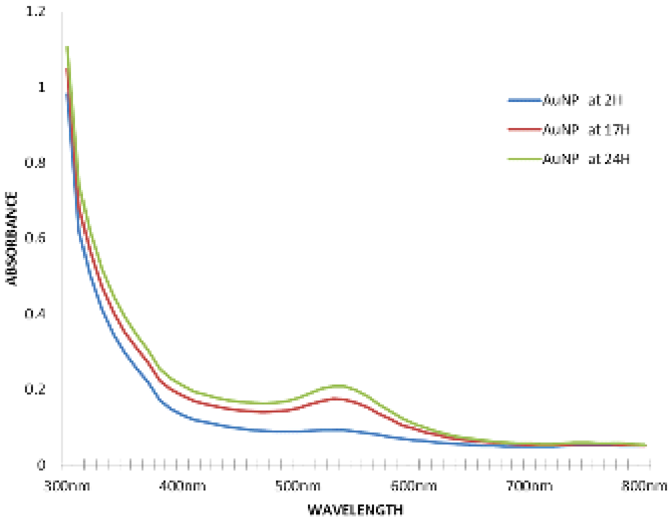

Fig. 2. The UV-Vis spectra of gold nanoparticles formed after different time periods

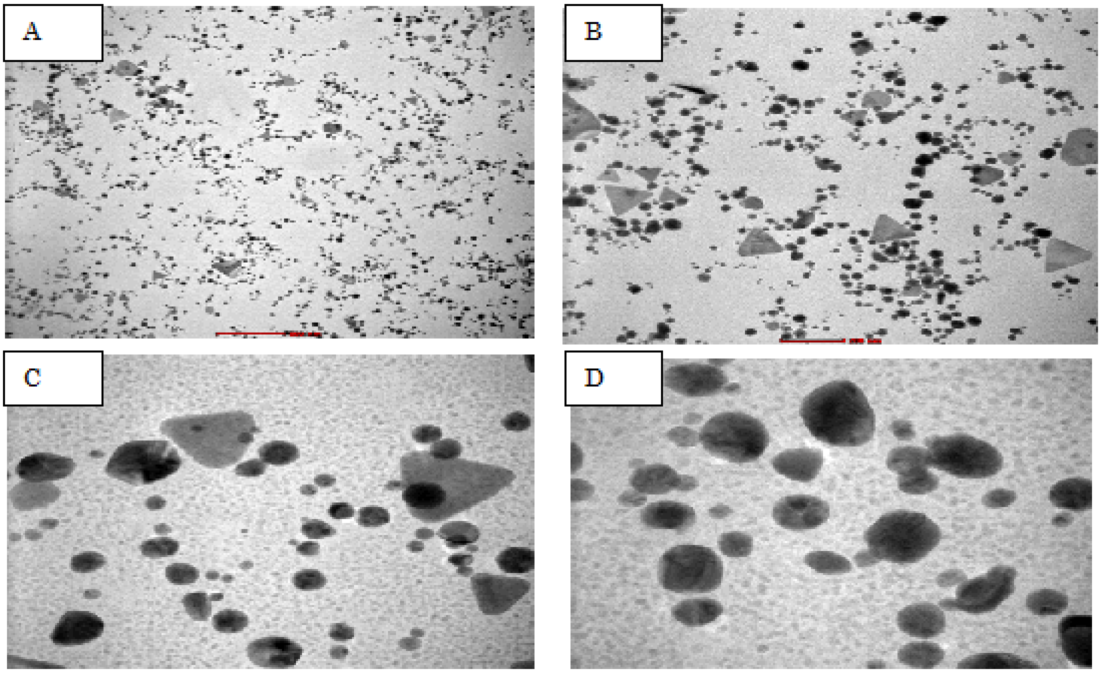

Fig. 3. TEM micrographs of gold nanoparticles synthesized using *Candida cylindracea*

by the yeast acting as a stabilizing agent for the nanoparticles.

CONCLUSIONS

The biological process for the formation of gold nanoparticles using *Candida cylindracea* has been demonstrated. This method is less costly, simpler, and requires less energy and raw materials than existing chemical methods. The development of an eco-friendly process for the synthesis of metallic nanoparticles constitutes an important step in the field of nanotechnology.

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