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ORIGINAL ARTICLE

In vitro Bionics of Face Centered Cubic Lattice Crystal Nanoparticles by *Saccharomyces cerevisiae* and Its Microbicidal Screening

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Abstract Extracellular synthesis of nanoparticles has received attention due to its more advantageous form of synthesis in large quantities and also easy for downstream processing. In the present investigation, we report *in vitro* extracellular synthesis of silver nanoparticles (AgNPs) using *Saccharomyces cerevisiae* with silver nitrate solution. The AgNPs were produced in 72 h of incubation time. The AgNPs formed were further characterized by means of UV-Vis spectrophotometry, Fourier Transform Infra-Red (FTIR), X-Ray Diffraction (XRD), and Scanning Electron Microscope (SEM). The synthesized AgNPs had maximum absorbance at ~420 nm in the UV- visible region. FTIR bands were observed at 3353.40, 2075.09, 1637.71 and 1397.30 cm^{-1}. XRD patterns of the AgNPs confirmed the formation of face centered cubic (fcc) lattice crystal silver particles. The SEM analysis revealed that the size of the AgNPs were of 30–60 nm. The synthesized AgNPs inhibited the growth of the test microorganisms at the concentration of 100 μL. The present study highlights the possibility of using the common baker's yeast *S. cerevisiae* to synthesize AgNPs and as a microbicidal agent.

Keywords Antmicrobial activity · Biosynthesis · Extracellular · Nanoparticles · *Saccharomyces cerevisiae* · Silver ions

Introduction

Nanotechnology is expected to take over both science and technology in upcoming decades. Engineered nanoparticles (ENPs) smaller than 100 nm have received more attention and concern recently due to their rapidly increasing applications in various areas of the economy, such as textiles, electronics, pharmaceuticals, cosmetics and environmental remediation (Royal Society, 2005; Dunphy-Guzman et al., 2006). Nanoparticles of free metals have been extensively studied because of their unique physical properties, chemical reactivity and potential application in catalysis, biological labeling, biosensing, drug delivery, antibacterial activity, antiviral activity, detection of genetic disorders, gene therapy, and DNA sequencing (Govindaraju et al., 2008). An important aspect of nanobiotechnology concerns with the development of experimental procedures for the reproducible synthesis of nanomaterials of controlled size, polydispersity chemical composition and shape (Sastry et al., 2004). Synthesis of nanoparticles can be achieved by various physical and chemical methods, but these methods are still in developmental stage and various problems are often experienced with the stability of the nanoparticle synthesis, control of the crystal growth and aggregation of the particles (Gericke and Pinches, 2006a). The achievement of clean, nontoxic, environmental friendly "green chemistry" procedures for the synthesis and assembly of nanoparticles both intracellularly or extracellularly by using the bacteria, fungi, actinomycetes, algae, yeasts and plants are possible. Among the eukaryotes, yeasts have been employed mostly in the biosynthesis of the semiconductor crystals or quantum semiconductor crystals. The yeast *Candida glabrata* synthesized monodispersed spherical-shaped peptide-bound CdS quantum crystallites of 20 Å (Dameron et al., 1989). Wurtzite typed hexagonal lattice-structured CdS nanoparticles in the range of 1–1.5 nm were produced by *Schizosaccharomyces pombe* (Kowshik et al., 2002a). Intracellular synthesis of Phosphate Buffered Saline (lead sulfide) nanocrystallites exhibiting quantum semiconductor properties was observed in yeast *Torulopsis* sp. (Kowshik et al., 2002b). The synthesis of nanosized AuNPs have been reported using common bakers yeast *Saccharomyces*

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cerevisiae (Lin et al., 2005), *Pichia jadinii* (*Candida utilis*) (Gericke and Pinches, 2006 a,b) and by the tropical marine yeast *Yarrowia lipolytica* NCIM 3589 (Agnihotri et al., 2009). Spherical shaped face centered cubic unit cell antimony oxide (Sb_20_3) nanoparticles in the size of 2–10 nm was observed by Jha et al. (2009) when they employed *S. cerevisiae*. Extracellular synthesis of the hexagonal shaped 2–5 nm sized AgNPs was reported in yeast strain MKY3 by Kowshik et al. (2003). With this background we have employed common baker's yeast *S. cerevisiae* to evaluate the efficiency for the synthesis of AgNPs.

Materials and Methods

Biomass fermentation. Biomass of the common baker's yeast, *S. cerevisiae*, was prepared under aerobic condition. The growth medium (100 mL) contained (g/l) tryptone- 2.0; yeast extract- 1.0 and glucose- 2.0 (Kowshik et al. 2003) in a 250 ml flask and was incubated in an orbital shaker at 25°C (150 rpm) for 72 h.

Synthesis and characterization of AgNPs. The cell free medium was filtered using Whatman no. 1 filter and the filtrate amended with 1mM $AgNO_3$ and incubated on an orbital shaker (150 rpm) at 25°C at for 72 h in a dark condition. After 72 h incubation, sample was withdrawn and the UV- Vis spectrum (200–800 nm) was measured using UV-Vis spectrophotometer (Optizen 2120UV, Seoul, Korea). The filtrate amended with $AgNO_3$ was used for the characterization of synthesized nanoparticles after lyophilization (Christ, ALPHA 1–2/LD plus, Germany) by Fourier transform infrared (FTIR) spectroscopy (Perkin Elmer RXI, USA) in the region of 4,000–400 cm^{-1}. X-Ray Diffraction (Rigaku D/Max ULTIMA11, Japan) analysis was recorded from 0 to 1400 at 2θ angles. The pattern was recorded by Cu L radiation with 1.5418 Å and graphite monochromatic filtering wave at tube voltage of 40 kV and tube current of 30 mA and scanning region of 0–70 at 6 min^{-1} with incident beam. To understand the distribution of synthesized AgNPs, the freeze dried powdered filtrate were mounted on the scanning electron microscopy (Hitachi S-3000, Tokyo, Japan) specimen stubs with double sided carbon adhesive tape than coated with platinum in a sputter coater at pressure of 8 pascal/20 mÅ for 90–120 seconds and the sample was examined at 12–15kV with a tilt angle 45° at magnification range of ×10k.

Antimicrobial assay. The antimicrobial activity of AgNPs was tested against the fungus such as *Aspergillus flavus*, *Aspergillus niger, Aspergillus oryzae*, and *Candida albicans*. Bacteria such as *Staphylococcus* sp., *Bacillus* sp., *Pseudomonas* sp., and *E. coli*. For antifungal activity, 0.1mL of fungal spores was aseptically added into Czapek-Dox agar plates. Cavities of 5mm were made and were filled with 25, 50, 75 and 100 µL of AgNPs. The plates were incubated at 28°C for 7 days. The antibacterial activity was carried out with 24 h active cultures of the chosen bacterial strains. The bacterial strains were seeded into nutrient agar medium. Central cavity of 5mm size was made in plates and filled with 25, 50, 75 and 100 µL of AgNPs. The plates were incubated in an incubator at 37°C overnight. The cavities filled with double distilled water served as control.

Results and Discussion

UV-vis spectrophotometer characterization of AgNPs. The filtrate without silver ions (control) did not show any colour change and the medium with silver ions turned brown after 72 h of incubation. It was an indication of formation of colloidal silver particles in the medium and it was inferred that the silver ions exhibited brown color in the aqueous solution. This color arises due to excitation of Surface Plasmon Resonance (SPR) in the metal nanoparticles (Ahmad et al., 2003; Basavaraja et al., 2008; Kumar et al., 2012). The formation of AgNPs depends on the dielectric constant of the medium and particle size (Balaji et al., 2009). The confirmation of formation and stability of the AgNPs in the aqueous solution was monitored by UV-Vis spectrophotometer. The supernatant of the yeast cell treated with silver ions showed a characteristic surface plasmon resonance band at ~420 nm after 72 h of incubation (Fig. 1). The resonance band at ~420 nm is due to the formation of homogenous spherical AgNPs (Vigneshwaran et al., 2007).

FTIR characterization of AgNPs. The FTIR spectrum was recorded from the freeze dried powder of AgNPs, formed after 72 h of incubation with filtrate. The amide linkages between the amino acids residues in the protein elucidate the well known signatures in the infrared region of the electromagnetic spectrum. The bands were observed at 3353.40, 2075.09, 1637.71, and 1397.30 cm^{-1} (Fig. 2). The bands 3353.50 cm^{-1} was assigned to the stretching vibrations of primary amine NH (Shaligram et al., 2009), similarly the band observed at 2075.09 cm^{-1} corresponds to the secondary amines NH. The band observed at 1637.71 cm^{-1} was assigned as stretch vibrations of C=C (Huang et al., 2007) and the band at 1397.30 cm^{-1} was due to the residual of NO^{3-} (Luo et

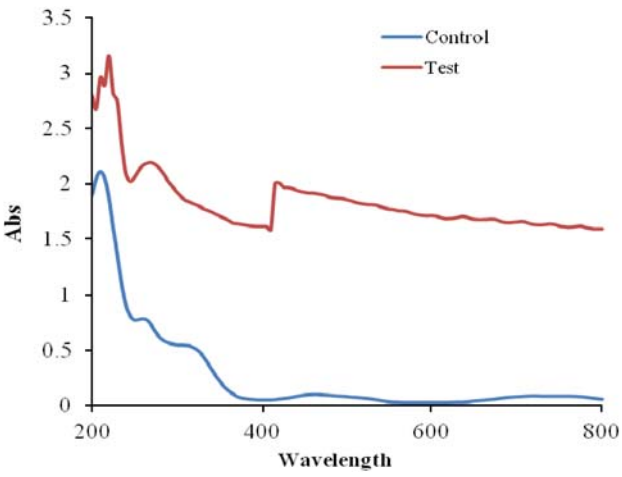

Fig. 1 UV-Vis spectra of filtrate amended with $AgNO_3$ as a function of time

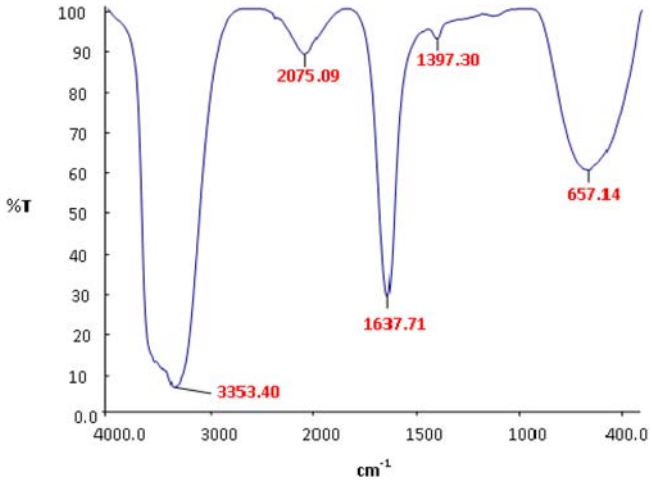

Fig. 2 FTIR spectra of freeze-dried powder of AgNPs formed after 72 h incubation of filtrate of *S. cerevisiae* with 1mM $AgNO_3$

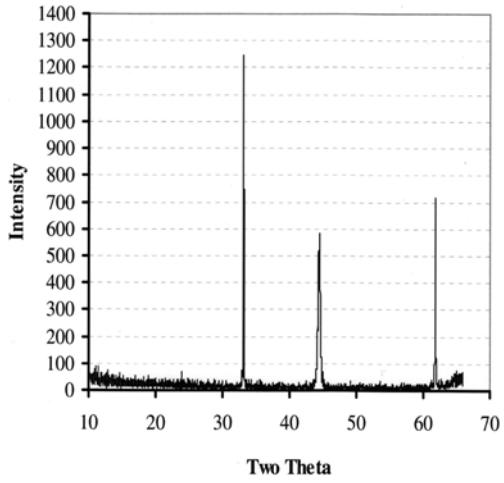

Fig. 3 XRD patterns of freeze-dried powder of AgNPs formed after 72 h incubation of filtrate of *S. cerevisiae* with 1mM $AgNO_3$

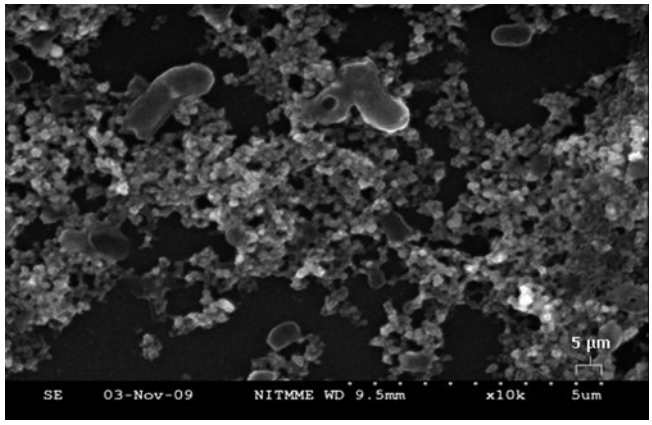

Fig. 4 Scanning Electronic Micrograph of the freeze-dried powder of AgNPs formed after 72 h incubation of filtrate of *S. cerevisiae* with 1mM $AgNO_3$

Table 1 Inhibition of activity of synthesized AgNPs on fungi

Amount (in µL)	Zone of inhibition (cm)			
	A. flavus	*A. niger*	*A. oryzae*	*C. albicans*
25	0.3	0.1	0.7	0
50	0.8	0.5	1	0.2
75	1.3	1	1.6	0.6
100	1.9	1.2	2	0.9

Table 2 Inhibition of activity of synthesized AgNPs on bacteria

Amount (in µL)	Zone of inhibition (cm)			
	Staphylococcus sp	*Bacillus* sp	*Pseudomonas* sp	*E. coli*
25	0.3	0.5	0.8	0.1
50	0.5	0.7	1.1	0.3
75	0.8	1.3	1.5	0.5
100	1	1.5	1.9	0.8

al., 2005). The IR observation confirms the presence of protein in the samples of AgNPs. Earlier reports states that the protein may bind to the nanoparticles either through free amine groups or cysteine residues in the proteins (Gole et al., 2001; Mandal et al., 2005) and through the electrostatic attraction of negatively charged carbohydrate groups in enzymes present in the cell wall of mycelia (Sastry et al., 2004) and as a result the AgNPs stabilization by the proteins are possible.

XRD patterns of AgNPs. The XRD analysis was done to support the results of UV-Vis spectrum and confirm the crystalline nature of the particle. The XRD pattern shows intense diffraction peaks at ~33°, ~45° and ~62°, 2θ (Fig. 3) which may be indexed to the (111), (200) and (220). This confirms the face-centered cubic (fcc) crystalline structure of silver. The diffraction peak corresponding to the (111) plane is more intense than the other planes. The intensity ratio between (200) and (111) diffraction is much lower than the visual value and it infers that the (111) plane is predominant orientation (Kannan and John, 2008).

SEM characterization of AgNPs. A SEM result gives a characteristic three-dimensional appearance which is useful for understanding the size and surface structure of a sample. The images of the SEM results are shown in Fig. 4. The AgNPs synthesized were spherical in shape and has a large distribution of sizes in the range of 30–60 nm. The synthesized AgNPs did not show direct contact within aggregates and the stabilization of nanoparticles occurs through a capping agent.

Inhibitory activity of nanoparticles on fungi and bacteria. The mode of action by the AgNP's could be the inhibition of the microbial processes on the cell surface and in the cell. Previous research demonstrated that AgNP's attach to the surface of cell membrane, affecting membrane permeability, dissipation of the ATP pool and Proton Motive Force (PMF) and finally caused cell death. The face centered cubic lattice crystal AgNPs inhibited the growth of the all the four fungi which was seeded in the agar plate

and shown a zone of inhibition around the central cavity. The highest zone of inhibition was observed under 100 µL of AgNPs with diameter of 2 cm was observed in respect of *Aspergillus oryzae*, followed by 1.9, 1.2, and 0.9 cm of *Aspergillus flavus*, *Aspergillus niger* and *Candida albicans,* respectively (Table 1). According to Dorau et al. (2004) the inhibition activity is due to the formation of insoluble compounds by inactivation of sulfhydryl groups in the fungal cell wall and disruption of membrane bound enzymes and lipids which causes cell lysis. The AgNPs also showed the antibacterial activity against *Staphylococcus* sp., *Bacillus* sp., *Pseudomonas* sp., and *E. coli* and formed zones of inhibition of diameters 1.0, 1.5, 1.9, and 0.8 cm, respectively with 100 µL of AgNPs (Table 2). Similar observations were recorded by Malliszewska et al. (2009) with AgNPs from *Penicillium* sp. Several other researchers also have reported the possible inhibitory action of AgNPs on various bacterial strains. Morones et al. (2005) reported that AgNPs were preferentially bound and localized on the membrane of *E. coli* cells. The dissipation of the proton motive force of the membrane in *E. coli* occurs when nanomole concentrations of AgNPs were given (Lok, 2006). In addition, the pitting of the cell membranes by AgNPs causes an increase in permeability and results finally in cell death (Shahverdi et al., 2007). In this study we demonstrated extracellular synthesis of AgNPs by *S. cerevisiae*. The characterization by UV-Vis spectrophotometer, FTIR, XRD, and SEM analyses confirmed the reduction of silver ions and formation of face-centered cubic AgNPs. The synthesized AgNPs were stabilized due to capping of proteins synthesized by the yeast. This is a simple, stable, environmental friendly, and easy to handle in downstream processing for large scale production. Antimicrobial assay also supported the possible application of synthesized AgNPs in biomedical application.

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