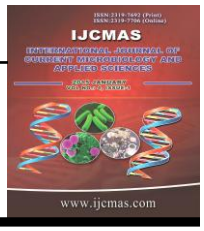
Int.J.Curr.Microbiol.App.Sci (2015) 4(3): 1084-1091

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 3 (2015) pp. 1084-1091**
http://www.ijcmas.com



Original Research Article

Biosynthesis of Silver Nanoparticles from Dental Caries Causing Fungi *Candida albicans*

K. Saminathan*

Centre for Nano Science and Technology, Tiruchengode, TN, India
**Corresponding author*

Keywords

Silver nanoparticles, *Candida albicans*, Pathogens, Antimicrobial activity

A B S T R A C T

The work is being carried out for the applications which would help in the prevention of human pathogens. We describe the synthesis of silver nanoparticles using fungi *Candida albicans*. A strong characteristic absorbance peak at around 400 nm was observed at different time intervals. Particle size analysis of these particles shows that they are 50-100 nm in range and also this paper deals with a thorough investigation on the characterization of the silver nanoparticles by UV Visible, XRD, SEM and FTIR analysis and result reveals that, the average grain size of the silver nanoparticles formed in the bioreduction process is determined by XRD pattern of the silver nanoparticles formed in the experiment and is estimated to be greater than 50 nm and spherical in shape. FTIR spectral analysis showed array of absorbance bands in 400 cm^{-1} -1500 cm^{-1} finally tested the biocompatibility by antimicrobial test against multi drug resistant human pathogens like *Escherichia coli*, *Klebsiella sp*, *Salmonella sp*, *Pseudomonas sp* and *Staphylococcus aureus*. The evaluation of agar diffusion test was made on the basis of zone of inhibition of bacteria around the test sample. The result reveals that the maximum antimicrobial activity was observed for *Staphylococcus aureus.*

Introduction

The biosynthesis of nanoparticles as an emerging highlight of the intersection of nanotechnology and biotechnology has received increasing attention due to a growing need to develop environmentally-benign technologies in material synthesis. This is not surprising given that many organisms, both unicellular and multicellular, are known to produce inorganic materials by either intra- or extra-cellular (Lovley *et al.*, 1987; Spring *et al.*, 1995; Dickson *et al.*, 1999 and Kathireswari et al 2014). In fact, microbes generally have a harder time developing resistance to silver than the do to antibiotics (Baker er al., 2005 and Landsdown and Williams 2007.,) The use of eukaryotes, especially fungi, is potentially exciting since they secrete large amounts of proteins, thus increasing productivity, and are simple to deal with in the laboratory. Moreover the process can be easily scaled up, economically viable with the possibility of easily covering large surface areas by suitable growth of mycelia. Furthermore, downstream processing would be much simpler using fungi (Mukherjee *et*

al., 2001). The use of fungi in the synthesis of nanoparticles is a relatively recent addition to the list of microorganisms. However, a novel biological method for the intra- and extra-cellular synthesis of silver nanoparticles using the fungi, *Candida albicans* has been documented. This has opened up an exciting possibility wherein the nanoparticles may be entrapped in the biomass in the form of a film or produced in solution, both having interesting commercial potential.

Materials and Methods

Dental caries swabs were collected from K.S.R dental campus, Tiruchengode, Tamil Nadu, India and the sterile cotton swabs were used for collection of samples. Immediately the samples were placed in the sterile ependroff tube. The collected samples were transported to lab and were inoculated in 100 ml peptone water in the conical flask and from that 0.1 ml poured on to the PDA (Potato Dextrose Agar) plates and spread. Then the plates were incubated at 37°C. After incubation the colonies were picked and staining techniques were performed. From that it was conformed as *Candida albicans* by performing the germ tube test and the culture medium were prepared, sterilized and inoculated with fresh culture of the *Candida albicans*. The culture flasks were incubated at 30º C for 24 hours. After incubation time the cultures were centrifuged at 10000 rpm and their supernatants were used for further experiments.

Silver nitrate ($AgNO_3$) at concentration of 1mM was separately added to the reaction vessels containing supernatants. The reaction between supernatant and Ag+ions was carried out in the dark and bright condition. The bioreduction of Ag^+ in aqueous solution was monitored by measuring the UV-Visible spectrum of the reaction medium at different time interval and different nanometer (280-580) by using a Perkin-Elmer Lamda-25 spectrophotometer. The particle size of *Candida albicans* synthesized nanoparticles has been obtained by the dynamic light scattering technique of laser light using particle size analyzer and to know the size and shape of the silver nanoparticles, it subjected to characterization. Due to our interest to get much smaller particles, above solution was centrifuged at a rate of 25000 rpm for 15 minutes and supernatant was air dried under hot air oven. The dried silver nanoparticles were subjected to SEM and FTIR analysis. The chemical groups present in the nanoparticles have been studied using FTIR (Perkin Elmer, USA).The air dried nanoparicles were coated on XRD grid and analyzed for the formation of Ag nanoparticles by Philips X-Ray Diffractometer.

Silver nanoparticles synthesized using sample of *Candida albicans,* are tested for its potential antimicrobial activity against few human pathogens. To analyze the antimicrobial activity of the sample, the samples were subjected to Agar well Diffusion Techniques as described by (Agarry *et al*., 2005). Wells of 6 mm diameter were cut on sterile nutrient agar plates and swabbed with an overnight broth culture of the organism. Each well was loaded with 40µl the solutions in the following order: antibiotics, silver nitrate solution and silver nanoparticles of *Candida albicans,* and incubated at 37°C ± 0.2 C. Antimicrobial activity in terms of zones of inhibition (mm) was recorded after 24 h of incubation. The antagonistic action of silver nanoparticles of *Candida albicans*were tested against test organisms namely *Escherichia coli*, *Klebsiella sp*, *Salmonella sp*, *Pseudomonas sp* and *Staphylococcus aureus*.

Result and Discussion

The formation of silver nanoparticles in the solution of 1mM silver nitrate and sample of *Candida albicans*, was confirmed by change in the color to yellow after incubation with silver nitrate, while the controls retained the original color. (Fig.1).The brown color appears immediately after the addition of the sample *Candida albicans*, and the reaction is completed in about 3hrs. This makes the investigation highly significant for rapid synthesis of silver nanoparticles.

The bioreduction of Ag^+ in the sample was monitored by periodic sampling of the reaction mixture at regular intervals by using UV-Visible spectroscopic analysis. The silver nanoparticles exhibits yellow color in water and this arises due to excitation of surface plasmon vibrations in the metal nanoparticles (Mulvaney et al., 1996). The UV-Vis spectra recorded from the aqueous silver nitrate of *Candida albicans*. A strong characteristic absorbance peak at around 400 nm was observed at different time intervals. The brown color of the medium could be due to the excitation of surface plasmon vibrations, typical of the silver nanoparticles (Vigneswaran et al., 2007).

The silver nano particles reduced form of silver nitrate solution through bioreduction are clearly distinguishable owing to their size difference and it is measured that 50 - 100 nm in size and the particles are spherical in shape. This may be due to the reduction in liquid solution and some chelating action also available in the solution. Due to this Silver particle nucleation is higher than the particle agglomeration.

Fig.1a Control

b Biosynthesis of silver nanoparticles

Fig.2 Shows the UV Visible spectral analysis

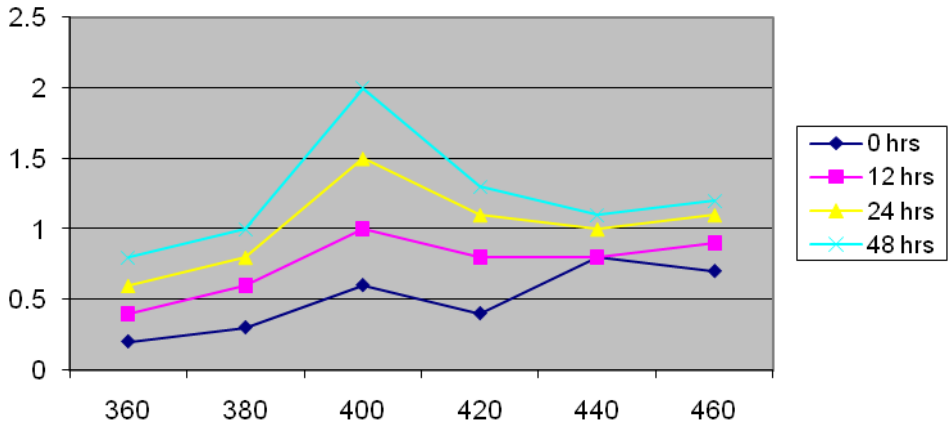


Fig.3 SEM image of the synthesized silver nanoparticles

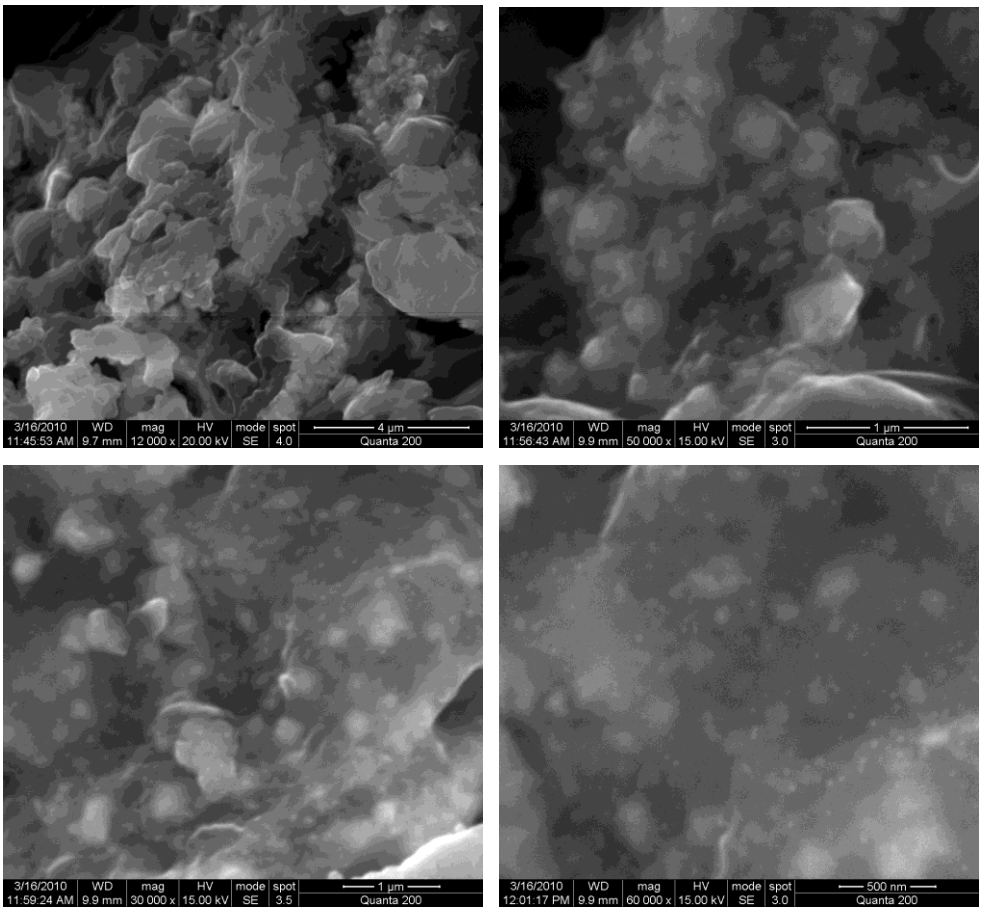


Fig.4 shows the particle size analysis of the synthesized silver nanoparticles

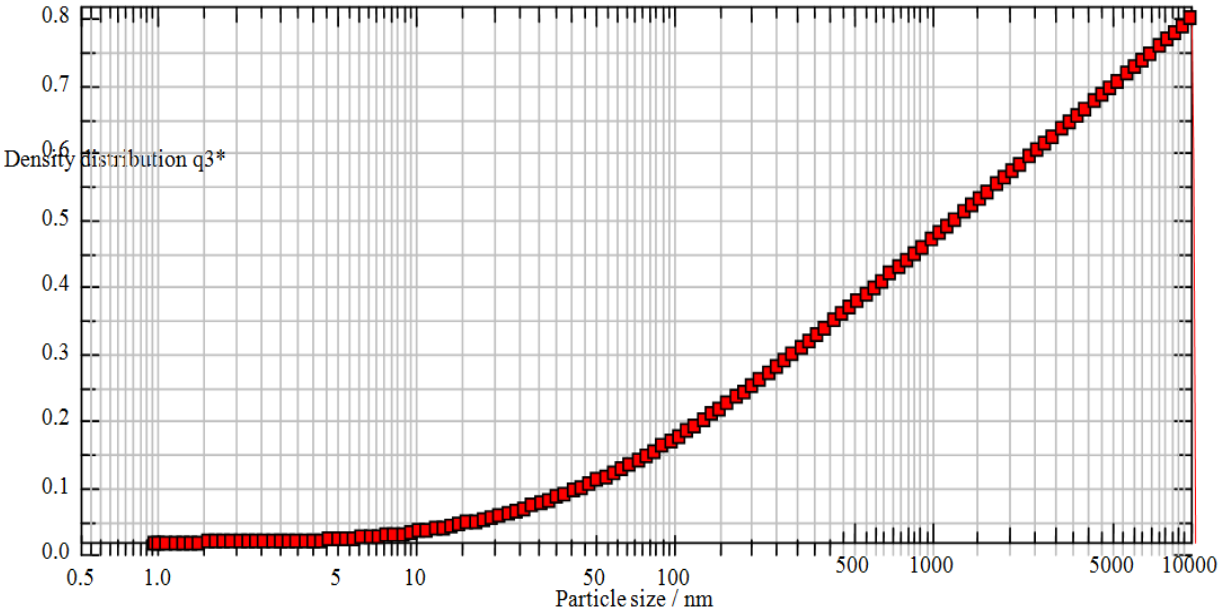


Fig.5 shows the XRD pattern of the silver nanoparticles formed in our experiment

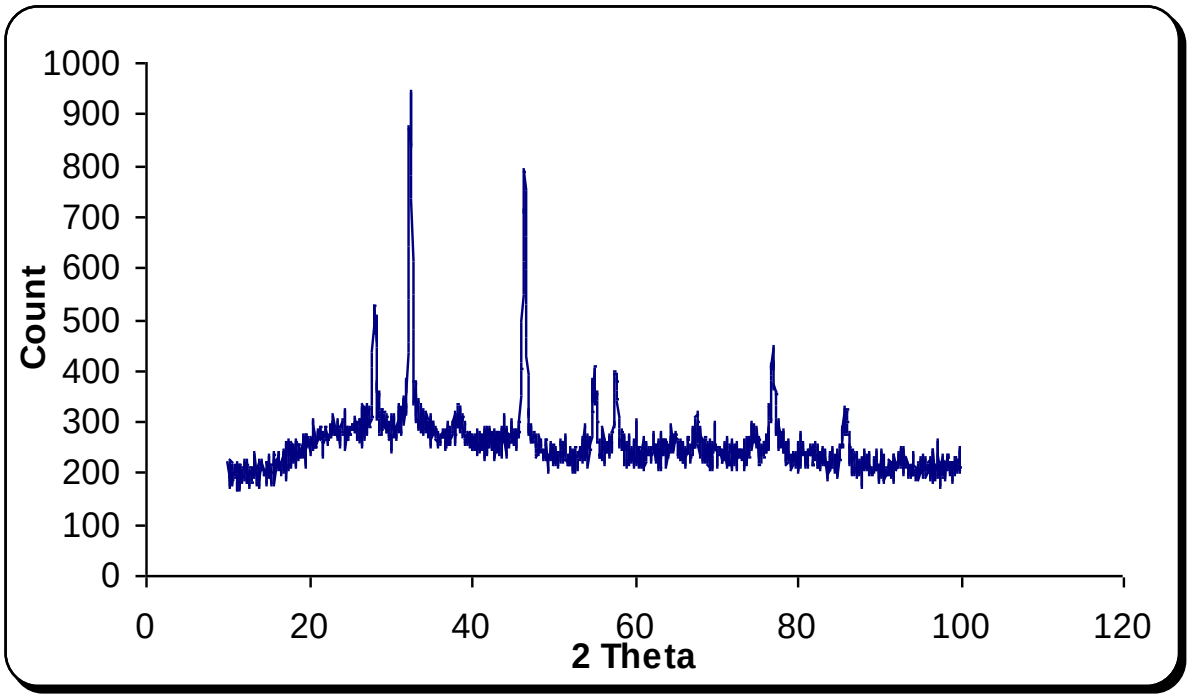


Fig.6 Show the FTIR analysis of synthesized silver nanoparticles by Candida albicans

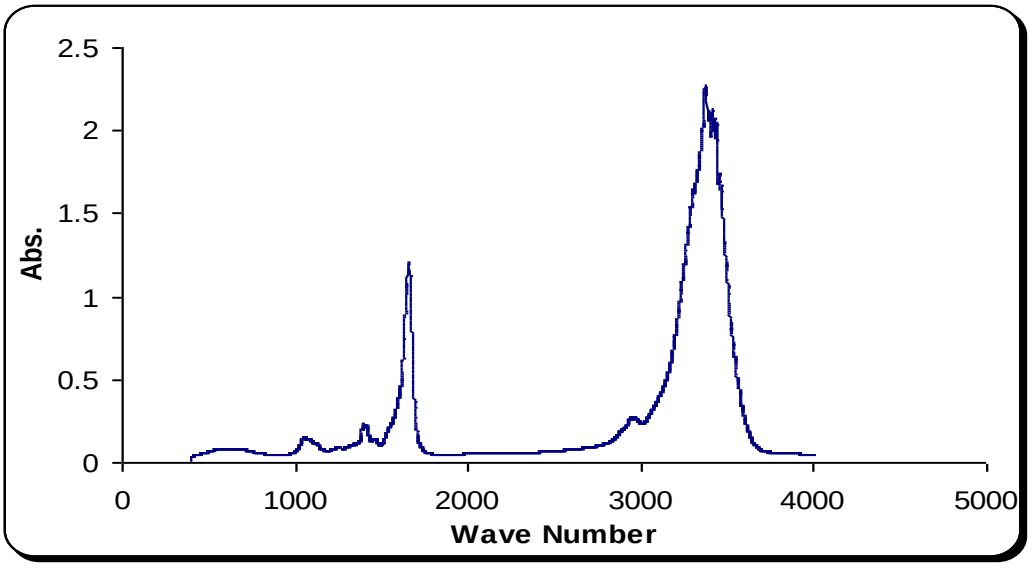
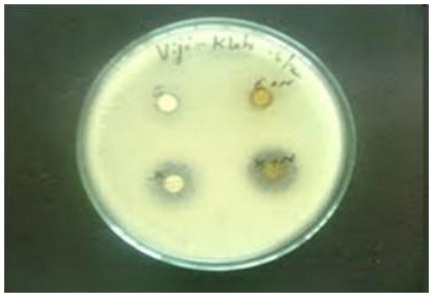
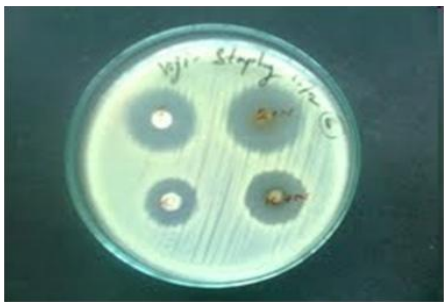
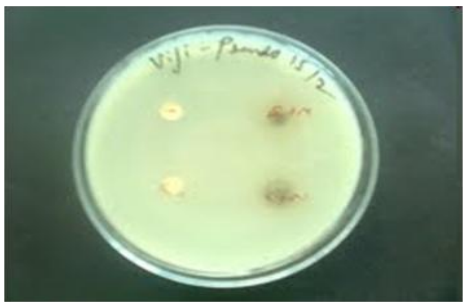


Fig.7 Antimicrobial activity of silver nanoparticles synthesized by Streptomyces sp



1. *Escherichia coli*, 2. *Klebsiellasp*, 3.*Salmonellasp*, 4. *Pseudomonas sp* 5.*Staphylococcus sp*





Particle size analysis

Particle size analysis shows that, when scanning from 1 nm, the particles count is very low and it's gradually increasing from reached the higher value at 20-100nm. So this indicates that the maximum nanoparticles in the range of 20 to 100 nm and few particles are present below and above this range (Fig. 4).

XRD analysis

X-Ray Diffraction studies of the sample shows three distinct diffraction peaks at 28.3˚, 32.4˚, 46.0˚ and can be indexed 2θ values of (220), (311), (420) crystalline planes of cubic Ag. The average grain size of the silver nanoparticles formed in the bioreduction process is determined using Scherr's formula, $d=(0.9\lambda*180^{\circ})/\beta \cos\theta\pi$ and is estimated to be 60 nm.

FTIR analysis

FTIR spectral analysis showed array of absorbance bands in 600 cm^{-1} - 4000 cm^{-1}. Organic functional groups are available in the air dried silver nanoparticles. The spectral bands were prominent at 3365 cm^{-1} (Amines), 1654 cm^{-1} (Alkenes), 1382cm^{-1} (Carboxylic acid). This organic group presence is due to Silver Particles reduction through biological sources. Fig. 5 shows the FTIR spectrum of the silver nanoparticles. Ultra pure Ag Particle can be prepared by removing the functional groups through chemical modification in future studies.

Antimicrobial activity of silver nano particles

Zone of Inhibition in the plate showed that silver nanoparticles synthesized using sample of *Candida albicans,* have the antibacterial activity against test pathogens namely *Escherichia coli*, *Klebsiella sp*, *Salmonella sp*, *Pseudomonas sp* and *Staphylococcus aureus*. On comparison with the antibiotics and *Candida albicans* synthesized silver nanoparticles well performed in the bactericidal effect (Fig 6).We have found that the silver nanoparticles synthesized in our study proposed study was effectively inhibited the growth and multiplication of pathogens like *Escherichia coli*, *Klebsiella sp*, *Salmonella sp*, *Pseudomonas sp* and *Staphylococcus aureus* on comparison with the antibiotics the synthesized silver nanoparticles out performed in the bactericidal effect.

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