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RESEARCH ARTICLE

Biosynthesis of Silver Nanoparticles Using *Saccharomyces Cerevisiae* with Different pH and Study of Antimicrobial Activity against Bacterial Pathogens

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Abstract: Extracellular biosynthesis of silver nanoparticles (Ag-NPs) using the *Saccharomyces cerevisiae* (Yeast) was carried out. The pH of the medium play a vital role in the synthesis of control shaped and sized nanoparticles. Morphological observation and characterization of biosynthesized silver nanoparticles were performed by UV-Visible spectroscopy, Scanning electron microscopy and Fourier transform infrared spectroscopy. The biosynthesized silver nanoparticles showed a maximum absorption in the visible region *Saccharomyces cerevisiae* strains showed a maximum absorption at 420-460 nm respectively and the size was ranged from 60-110 nm and 10-40 nm respectively. The antibacterial activities of silver nanoparticles (Ag-NPs) were studied with *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative). The silver nanoparticles were synthesized at pH 6 that showed maximum antibacterial activity. This method is a promising eco-friendly alternative to chemical method.

Keywords: *Saccharomyces cerevisiae*, Biosynthesis, Extracellular synthesis, Nanoparticles, Antimicrobial activity

Introduction

One of the most important criteria of nanotechnology is that of the development of clean, nontoxic and environmentally acceptable ‘‘green chemistry’’ procedures, involving organisms ranging from bacteria to fungi and even plants[1,2]. The interactions between microorganisms and metals have been well documented and the ability of microorganisms to extract and accumulate metals is already employed in biotechnological processes such as bioleaching and bioremediation.

It is known that a large number of organisms, both unicellular or multi cellular, are able to produce inorganic nanomaterials, either intracellularly or extracellularly. It seems that especially the yeast and fungi are very good candidates for the synthesis of silver nanoparticles because these types of biomasses are easily handled[3].

Yeast being a member of the class Ascomycetes also called sac fungi in kingdom fungi, it has been taken into regular use as media supplement in different culture procedures and this organism itself has been a very good source of different enzymes and vitamins. Eco-friendly approach for nanomaterials synthesis should not use toxic chemicals in the synthesis protocol. In the present effort, the baker's yeast (*Saccharomyces cervasae*) has been taken in order to assess its potential as candidate fungal genera for the transformation of silver nanoparticles[3].

In this study, the silver nanoparticles were synthesized by an extracellular synthesis process using *Saccharomyces cerevisiae* cell culture and then the effect of pH on the synthesis of silver nanoparticles was examined by changing the pH of the aqueous cell filtrate with 0.1 N sodium hydroxide and hydrochloric acid. The synthesized nanoparticles were characterized. The antibacterial activity of silver nanoparticles was examined against *E. coli* and *Staphylococcus aureus*

Experimental

Silver nitrate, nutrient agar, nutrient broth, luria bertani medium, sodium chloride, hydrochloric acid were obtained from Himedia Pvt.Ltd., India. Yeast (*Saccharomyces cerevisiae*) was isolated from graph juice. Pathogens *Staphylococcus aureus* and *Escherichia coli were* isolated from clinical samples**.**

Extracellular synthesis of silver nanoparticles

Saccharomyces cerevisiae was inoculated at 0.5% level in 2 L Erlenmeyer flasks containing 1L growth medium (2% tryptone, 1% yeast extract and 2% glucose, pH 5.6). The flasks were incubated at 30 ^{o}C on a rotary shaker set at 100 rpm. Upon attaining the mid-log phase (between 9 and 10 h, O.D.600=2), the cells were separated from the culture medium by centrifugation (5000 rpm) and the cell-free medium was used for the recovery of precipitated silver nanoparticles. 1 mM of silver nitrate was added to the cell-free medium to the synthesis. The silver nanoparticles were incubated further in dark for 24 h. The UV absorption spectrophotometer reading was taken at different time intervals to monitor the synthesis of silver nanoparticles extracellularly.

Effect of pH on the extracellular synthesis of silver nanoparticles

The influences of pH on the extracellular synthesis of silver nanoparticles were carried out by changing the pH of the cell-free medium. The different pH was taken (4 and 6) to examine the effect of pH on the synthesis of silver nanoparticles using *Saccharomyces cerevisiae*. The pH of the cell-free medium was changed using 0.1 N hydrochloric acid and 0.1 N sodium hydroxide. UV spectrophotometer was used to take the absorption at 24 h of incubation.

Characterization of biosynthesized silver nanoparticles

The UV absorbance spectra were taken at various time intervals at different wavelength. Scanning electron microscope used to identify the shape of the synthesized silver nanoparticles. The functional groups of biologically synthesized dried nanoparticles observed using Fourier Transform Infrared Spectrometer.

Antibacterial activity of silver nanoparticles

The antibacterial activity of biosynthesized silver nanoparticles was carried out against *Staphylococcus aureus* and *Escherichia coli.* Various concentrations (10 µL, 20 µL, 30 µL, 40 µL and 50 µL) of silver nanoparticles were synthesized with different pH (4 and 6). The well-diffusion method was used to determine the antibacterial activity of silver nanoparticles.

The well was formed in the medium using cork borer. The silver nanoparticles were pipette out into the wells, and then the plates were incubated at 37 ^{o}C for 24 h. After 24 h of incubation, the plates were observed for the zone of inhibition.

Results and Discussion

Extracellular synthesis of silver nanoparticles

The preliminary confirmation for the formation of silver nanoparticles was the visual observation of colour change of the aqueous solution of yeast culture. Before the addition of silver nitrate the culture was in yellow colour. After the addition of silver nitrate, the extracellular culture colour changed to white precipitate and at 24 h of reaction, the colour of the solution changed to brown (Figure 1). Synthesized silver nanocrystals using *Sacharomyces cerevisiae* they obtained the similar colour changes during the formation of silver nanoparticles[4].

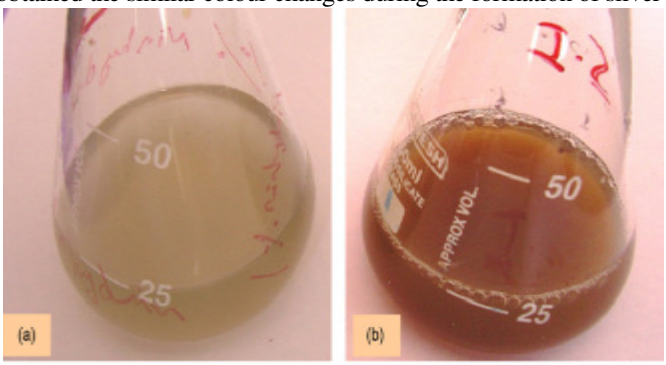

Figure 1. Culture filtrate with silver nitrate solution at the (a) beginning of the reaction and (b) after 24 h of reaction

Characterization of biosynthesized silver nanoparticles

UV Absorption spectrum

The UV absorption spectral studies were carried out to confirm the formation of silver nanoparticles using *Sacharomyces cerevisiae*. Figure 2 shows the peak found at 420 nm. The peak appears very broad with number of sub peaks and shoulders and it is proposed that such a broad peak indicates that there exists a wide range of nanoparticle sizes.

Figure 3 shows the effect pH on the synthesis of silver nanoparticles at pH 6. The maximum production of silver nanoparticles occurred. The absorption peak occurred at 420 nm and 460 nm for pH 6 and pH 4 respectively. The band at 420 nm indicated the spherical shape of nanoparticles, whereas at 480 nm the particles are different shapes[5].

SEM

Silver nanoparticles were synthesized using pH 4. The synthesized particles were hexagonal in shape and the size of the nanoparticles was in the range of 60-110 nm. Figure 4a and Figure 4b shows the SEM images of the silver nanoparticles synthesized using pH 6. The particles were spherical and the obtained particles are 10-40 nm in size. The size of nanoparticles is high at acidic pH, because the nucleation process for the formation of silver nanocrystal at acidic pH is slow. The low amount of large size particles were formed. While at high pH, more nucleation process occurred because of the accessibility of –OH ions. Thus high amount of small size particles formed[6].

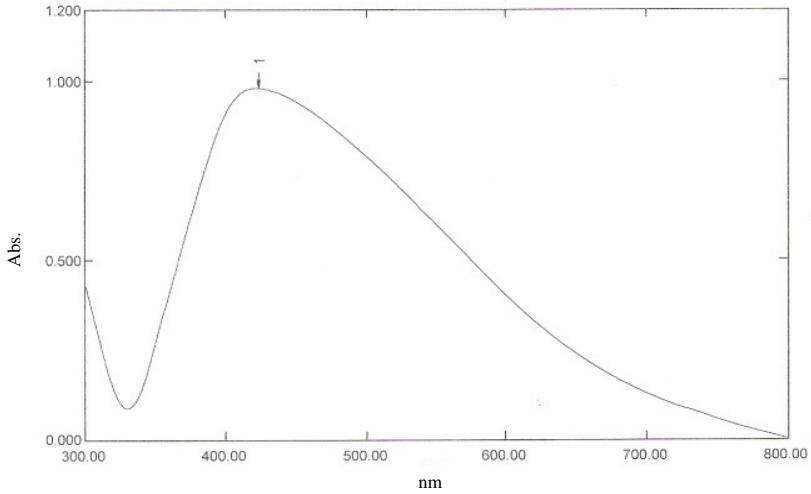

Figure 2. UV- Absorption spectrum, the peak found at 420 nm the peak appears very broad with number of sub peaks and shoulders and it is proposed that such a broad peak indicates that there exists a wide range of nanoparticle sizes

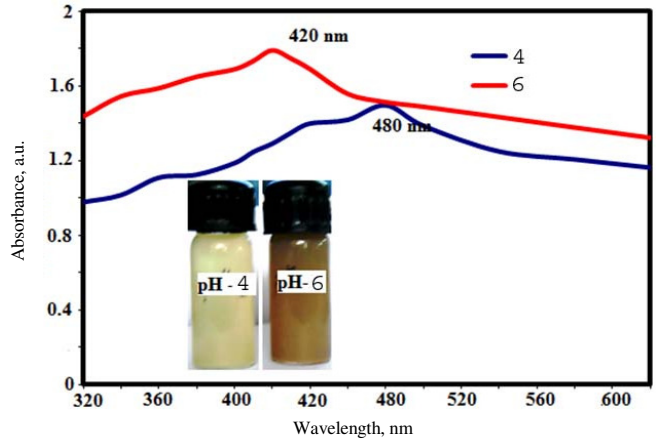

Figure 3. UV-Spectrophotometer absorption of the effect of pH on the synthesis of silver nanoparticles. Inset shows the colour variation at pH-4. The synthesis of silver nanoparticles is low and at pH-6 the production of silver nanoparticles is high

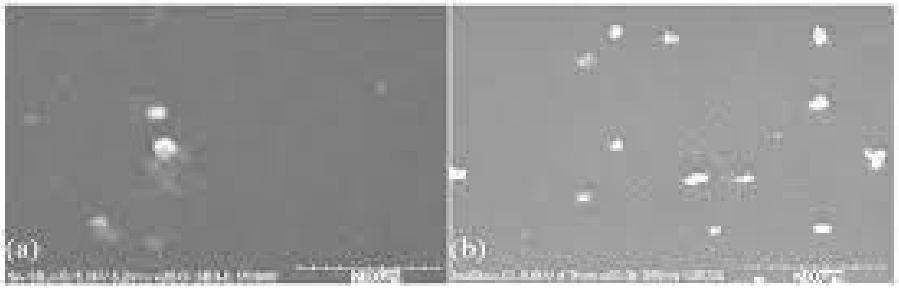

Figure 4. SEM image of the synthesized silver nanoparticles (a) pH 5, (b) pH 9

FTIR

The FTIR spectrums of silver nanoparticles were synthesized using *Sacharomyces cerevisiae* (Figure 5). The band at 3412 cm^{-1} and 2918 cm^{-1} represent the O-H, C-C stretching vibration[7]. The band at 1634 cm^{-1} represents the –NH stretching vibration of the amide group[8]. The bands at 1381 cm^{-1} and 1058 cm^{-1} represent the aromatic and aliphatic amines of C-N stretching vibrations of protein[9]. The FTIR results confirmed that protein might be responsible for the formation of silver nanoparticles[10,11].

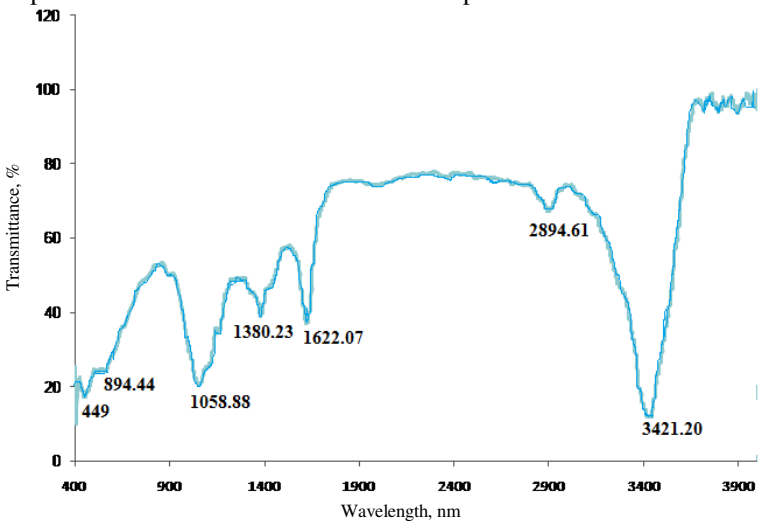

Figure 5. FTIR Spectrum of biosynthesized silver nanoparticles using *Saharomyces Cerevisiae*

Antibacterial activity

The well diffusion method was used to provide evidence for the antibacterial activity of biosynthesized silver nanoparticles against *E.coli* and *Staphylococcus aureus* Figure 6 and Figure 7 shows the antibacterial activity of silver nanoparticles synthesized using pH 4 and pH 6 against *E.coli* and *Staphylococcus aureus.* The antibacterial activity of silver nanoparticles indicated by the formation of the zone and the zone of inhibition measured as mm/diameter. The maximum zone of inhibition occurred at 50 µL concentration of silver nanoparticles. The silver nanoparticles synthesized using pH 6 show higher antibacterial activity against *E.coli* and *Staphylococcus aureus*[12,13].

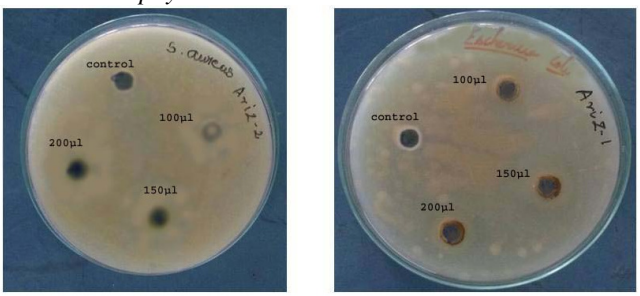

Figure 6. Antibacterial activity of biosynthesized silver nanoparticles synthesized using pH 4 against *E.coli* and *Staphylococcus aureus*

Figure 7. Antibacterial activity of biosynthesized silver nanoparticles synthesized using pH 6 against *E.coli* and *Staphylococcus aureus*

Conclusion

The silver nanoparticles were synthesized using *Saharomyces cerevisiae* by extracellular method. The different sized and shaped nanoparticles formed while changing the pH of the aqueous solution. The proteins which are present in the bacteria may be a possible reason for the synthesis of silver nanoparticles. The pH of the aqueous solution plays an important role in the antibacterial activity of silver nanoparticles. The smallest nanoparticles synthesized using pH 6 showed more antibacterial activity than large particles which are synthesized using original pH and pH 4.

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