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Antifungal Activity of Silver Nanoparticles Produced from Fungus, *Penicillium fellutanum* at Different pH

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Abstract

Currently there are many approaches being engaged in the production of silver nanoparticles however these methods uses reducing agents such as sodium borohydride, hydrazine and organic passivators like thiourea, thiophenol, mercaptoacetate, etc. which are environmental pollutants and increases the overall cost of the process. Therefore biosynthesis of silver nanoparticle is now considered to be the most environmental friendly, cost effective method. The effect of different pH on the synthesis of silver nanoparticles was observed by altering the pH of the fungal filtrate using 0.1 N sodium hydroxide and 0.1 N hydrochloric acid. Rapid rate of silver nanoparticles were obtained at pH 8.0 at λ_{max} of 440. Produced silver nanoparticles were effective antifungal agents against *Candida albicans, Candida glabrata* and *Candida tropicalis.*

Keywords: Nanotechnology; Antifungal; Disc diffusion method; *Candida albicans*

Introduction

Acidification, conjugation, capping, mineralization, use of biological entities such as enzymes for catalysis and bioreduction, etc. are the other commonly employed modes used for the production of nanoparticles [1,2]. Nanoparticle synthesis can be either extracellular (nanoparticles are forming outside the cell) or intracellular formation (nanoparticles are forming inside the cell) depending on the location where these nanoparticles are formed however the exact mechanism is not understood completely [3-5].

Extracellular assembly of nanoparticles is more advantageous of the fact that it makes the downstream processing for the recovery and purification of the product much easier and does not require lysis of the host cell [6]. In addition according to Sharma et al. uses of nanoparticles will be better analysis if produced extracellularly [7]. Intracellular process of nanoparticle synthesis offers easy optimization to obtain definite size and shape of the nanoparticles but recovery and purification from the biomass is a tedious task .it requires sophisticated equipment and expertise [6]. For example sulphate reductase contributes in sulphate ions reduction thus acting as a bioreducing agent and produces intracellular nanoparticles with semi conductive properties. While X-ray diffraction analysis depicts the nano crystalline nature of the nanoparticles.

Numerous publications have suggested that metallic nanoparticles have wide range of applications specifically in biomedical domains i.e. in nano medicine where they can be used in gene and drug delivery systems [8,9], in phagokinetic studies [10], in tissue engineering [11,12], in tumor demolition through heating (hyperthermia) [13], as fluorescent biological labels [14,15] in bio detection of pathogens [16] and in MRI contrast enhancement [17]. Among different nanoparticles, Nanosilver is the most mentioned nanomaterial and is evolving as the most effective nanoparticle with variety of applications [18]. Several examples stated in the literature that shows that the biosynthesis of silver nanoparticle can be achieved by the use of variety of organisms. For example extracellular production of stable silver nanoparticles using the fungus *Aspergillus flavus* has been reported by [18].

Similarly bacteria like *Brevibacterium casei* and Bacillus species [19,20] are well known producers of silver nanoparticles. In addition plants such as Curry leaf (*Murraya koenigii*) was reported to synthesize spherical shape silver nanoparticles of uniform size of 10-25 nm [21]. In another study by Qian et al, *Epicoccum nigrum*, an endophytic fungi was found to synthesize AgNPs at varied temperature and pH [22].

Penicillium fellutanum, a fungus usually associated with decaying matter. Besides producing different antibiotics are well known producers of nanoparticles of silver [5,9]. In this work $AgNO_3$ was fabricated at different pH from *Penicillium fellutanum* and evaluation of its antifungal activity was investigated against clinical pathogens.

Materials and Methods

Penicillium fellutanum, was grown in one liter of YM (yeast malt extract) broth prepared by dissolving peptone (5 g/l), yeast extract (3 g/l), malt extract (3 g/l), glucose (10 g/l) in 1000 ml of distilled water. The growth media was sterilized at 121°C for 20 min at 15 psi (pound/square inches). YM media was inoculated with *Penicillium fellutanum* and incubated for 120 h at room temperature on orbital shaker. After 120 h of growth the fungal filtrate was obtained by harvesting the fungal biomass using Whatman's filter paper no. 42 and saved for further use. The obtained cell free filtrate was then centrifuged at 10000 rpm for 20 min.

20 ml of supernatant at different pH incubated with 90 ml of 2 mM silver nitrate solution for 48 h in dark at room temperature. UV visible spectrometry absorbtion analysis recorded from 400 nm to 500 nm to determine λ_{max}. pH of the supernatant was altered using 0.1 N sodium hydroxide and 0.1 N hydrochloric acid to adjust the pH to 5.0, 6.0, 7.0, 8.0 and 9.0, respectively. Five test tubes each containing 20 ml

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of supernatant at different pH incubated with 90 ml of 2 mM silver nitrate solution for 48 h in dark at room temperature. 1 ml of the sample from each test tube was withdrawn after 48 h and subjected to UV-visible spectroscopy to measure its absorbance at a specific optimum wavelength to determine the optimum pH value of the extracellular aqueous media required for the production of silver nanocrystals. Distill water was used as reference.

Antifungal activity of silver nanoparticles was assessed using disc diffusion method carried on YM agar plates (dissolving yeast extract (3 g/l), glucose (10 g/l), peptone (5 g/l), malt extract (3 g/l) and agar (20 g/l) in 1000 ml of distilled water. The growth media was sterilized at 121°C for 20 min at 15 psi (pound/square inches)) Before pouring 0.5 ml antibiotic ampicillin was added to the medium to inhibit bacterial contamination and 1 ml of 10% sterilized tartaric acid solution to adjust pH of media (4.2-4.5).The surface of the agar was inoculated with the test pathogens such as *Candida glabrata, Candida albicans* and *Candida tropicalis.* Then sterile discs of size one centimeter was prepared and placed on agar surface impregnated with different concentration of silver nanoparticle solution (20 µL, 40 µL, 60 µL and 80 µL) in order to determine the dose dependent concentration .Incubation was done at 37°C for 48 h, caliper was used to measure the diameter of the inhibition zone. Silver nitrate solution impregnated discs were used as control.

Results and Discussion

The confirmation of silver nanoparticles in the fungal filtrate incubated with silver nitrate solution for 48hrs was by means of visual change in the filtrate color from deep yellow to grey color (Figure 1) .change in color means that silver metal ions has been reduced by nitrate reductase, a NADPH-dependent enzymes involved in the synthesis of silver nanoparticles [23,24]. Bioreduction of silver ions were carried out by reduction-specific enzymes which resulted in the formation of silver metal ion aggregates which in turn form the desired silver nanoparticle [25].

UV visible spectra depicted maximum absorption at 440 nm (Figure 2). Similar results were mentioned by Duran et al. in his work [23]. Silver nanoparticles produce an intense absorption and scattering.

Five test tubes containing fungal filtrate incubated with silver nitrate at different pH displaying different color intensities indicating silver nanoparticles synthesis (Figure 3).

UV visible spectra obtained at different pH at 440nm shows that maximum synthesis of silver nanoparticles occurred at alkaline pH of 8.0 (Figure 4). Thus, the synthesis of silver nanoparticles was greatly influenced by pH as confirmed by Riddin et al. that increased pH resulted in faster nanoparticle production [26]. The maximum peak at pH 8.0 indicates the formation of nanoparticles with a size range between 10

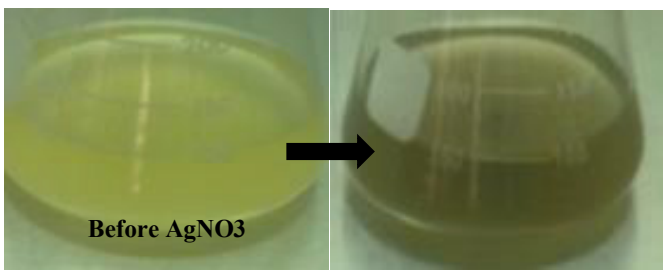

Figure 1: Appearance of grey brown color of the fungal filtrate after addition of silver nitrate solution.

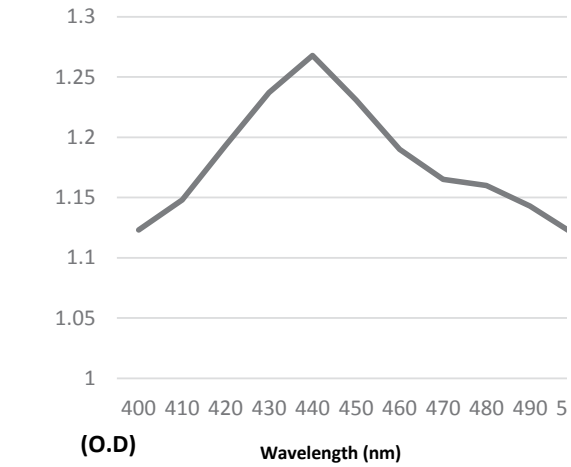

Figure 2: UV visible spectrum showing maximum absorbtion at 440 nm.

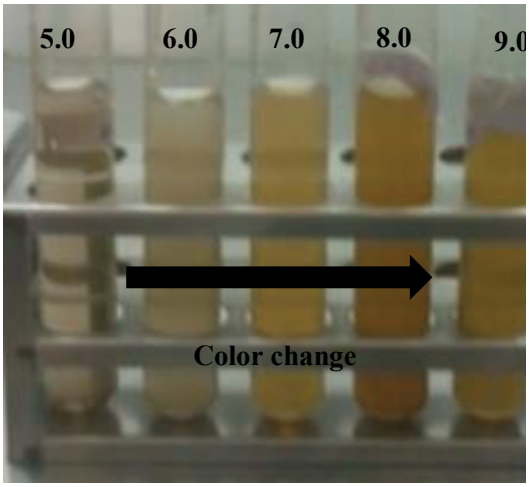

Figure 3: Color change in five test tubes at different pH indicating silver nanoparticles synthesis.

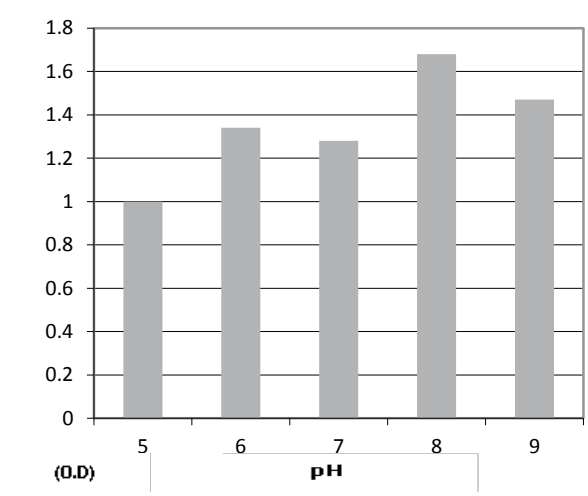

Figure 4: UV visible spectrum showing maximum silver nanoparticle synthesis at pH of 8.0.

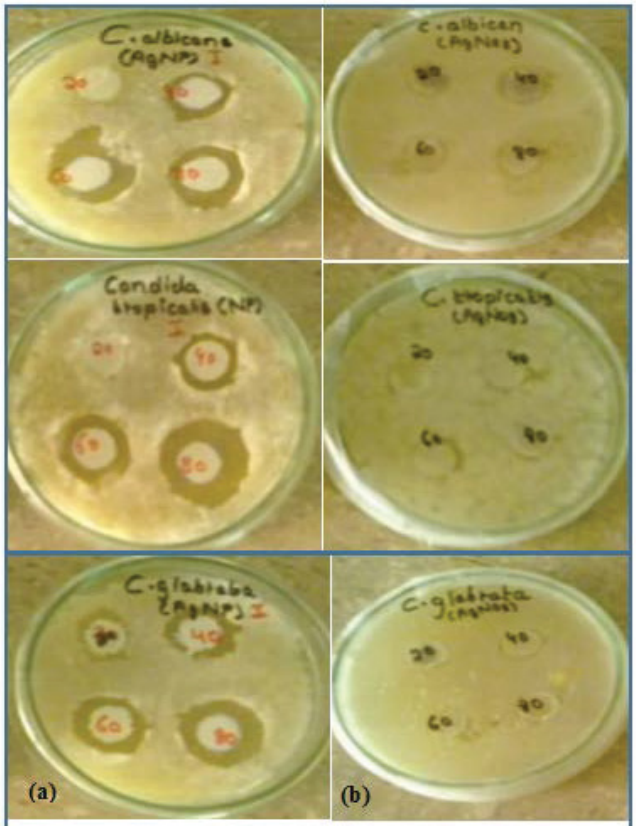

Figure 5: Antifungal activity of (a) silver nanoparticles synthesized at altered pH (b) silver nitrate (control).

to100 nm and showed maximum synthesis of silver nanoparticles. At alkaline medium number of functional groups available for silver binding increases thus facilitating higher number of Ag (I) to bind and subsequently results in the fabrication of large number of small sized silver nanoparticles with spherical morphology [27]. On the contrary decrease in pH to 5 did not show any peak. At low pH, protein active sites gets affected and denatured and loses its activity thus aggregation of nanoparticles was seen [28].

Silver nanoparticles possess increased antifungal activity against Candida species [28-30]. Results obtained from disc diffusion method indicates Silver nanoparticles as effective antifungal agents against as *Candida glabrata, Candida albicans* and *Candida tropicalis* however no inhibition was observed in controls (Figure 5).

Conclusion

Silver Nanoparticles produced from fungus, *Penicillium fellutanum* at different pH were found to possess effective antifungal activities against clinical pathogens.

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