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Synthesis of AgNPs using *Aspergillus terreus* and Evaluation of Its Antimicrobial Activity

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Abstract

Extracellular mycosynthesis of Ag Nano crystals by using the biological organism *Aspergillus terreus* is one of the most environmental friendly approaches as *Aspergillus terreus* can easily be cultured on simple media nutrients and shows high metal tolerance. Downstream processing for recovery of extracellular Ag nanocrystals is simpler making the process commercially feasible. Silver nanoparticles fabrication was characterized using UV-visible spectrophotometer and maximum absorbtion was obtained at 450 nm. Nitrate reductase test was used to confirm the presence of reductase protein involved in Ag nanoparticles fabrication. These mycosynthesized nanoparticles were found to have broad antimicrobial spectrum against pathogens including *C. albicans, C. tropicalls, C. parapsilosis, C. glabrata, C. krusei, A. flavus, E. coli, P. aeruginosa* and *S. aureus* as determined by agar diffusion method.

Keywords: *Aspergillus terreus*; Agar disc method; UV visible spectrophotometer; BUITEMS

Introduction

Nanoparticles of silver have been synthesized by a number of different organisms such as bacteria, fungi, algae, etc. For example synthesis of uniformly distributed AgNPs have been reported in *Escherichia coli* [1,2]. *Pediococcus pentosaceus, Lactococcus garvieae* and *Enterococcus faecium*, were used to produce AgNPs non-enzymatically through the interaction of organic compounds present on bacterial cell surface with silver ions. On the other hand *Lactobacillus* spp. depicts rapid synthesis of AgNPs for better silver nanoparticle recovery at high pH [3]. *Chlorella vulgaris* is unicellular green algae which is also reported to synthesize crystalline metal nanoparticles at room temperature. The carboxyl and hydroxyl groups in proteins are responsible for the metal ion reduction [4]. On the other hand *Trichoderma reesei* fungus, an eco-friendly fungus can be used for scale up production of silver nanoparticle due to its elevated capacity to produce high concentration of extracellular enzyme [5]. Large quantities of silver nanoparticle can be obtained from yeast strain MKY3 which is silver tolerant, with an average size range from 2-5 nm [6]. Similarly from extremophilic yeast strain AgNPs of average diameter 20 nm has been synthesized which were well dispersed and capped by proteins secreted by the yeast [7]. Thus synthesis of AgNPs using fungi has numerous advantages over other synthetic routes including environmental friendly and cost effective synthesis process with higher amounts of protein expression resulting in large scale production [8]. In current study synthesis of silver nanoparticles have been accomplished using *Aspergillus terreus* and evaluation of its antimicrobial activity was investigated against different pathogenic fungi and bacteria using agar disc method.

Materials and Methods

Fungal biomass production

Aspergillus terreus cultured was obtained from Yeast and Fungal Biotechnology Lab, Department of Microbiology, Faculty of life sciences, BUITEMS. *Aspergillus terreus* was grown aerobically in cezapex dox (CD) broth which consists of Calcium chloride (0.5 g/l), Glucose (10 g/l), Ferrous sulphate (0.01 g/l), Magnesium sulphate (0.5 g/l), Sodium nitrate (2 g/l), Yeast extract (1 g/l), Potassium dihydrogen phosphate (1 g/l) and zinc sulphate (0.01 g/l). All the fungal growth media components were dissolved in thousand mL of distilled water to make the solution up to one liter. Cezapex dox (CD) broth was than sterilized by autoclaving at 121°C for 20 min at 15 psi (pound/square inches). Inoculation of the media with *Aspergillus terreus* was done by wire loop method under laminar flow cabinet. The inoculated media was placed on an orbital shaker at room temperature at 120 rpm for five days.

Harvesting of fungal biomass

Fungal mycelia was obtained from the broth by simple filtration using Whatman's filter paper no. 42 to obtain cell free fungal filtrate (FF).The fungal filtrate was centrifuged at 15000 rpm for 10 min. Freshly prepared CD broth with aqueous silver nitrate was considered as the control (experiment and control were performed in triplicates).

Green synthesis of AgNPs

20ml of centrifuged fungal filtrate (FF) was incubated with 80ml of one millimolar $AgNO_3$ solution in an incubator at 25°C for 24 h in dark.

Confirmation of AgNPs synthesis

AgNPs synthesis was confirmed by visual change of the color of fungal filtrate and by means of UV visible spectrophotometer analysis.

Nitrate reductase assay

Fungal filtrate was subjected to nitrate reductase assay [9]. 2 mL of filtrate was mixed with 2 mL of the nitrate reductase assay medium which consists of 30 mM KNO_3 and 5% iso-propanol in 0.1 M phosphate buffer of pH 7.5 and incubated at 25°C for 1 h in dark .Then

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addition of 1 mL of 10 mM NEED (N-(1-naphthyl) ethylene diamine dihydrochloride) and 1 mL of 50 mM sulphanilamide) solutions were added. UV-Vis spectrophotometer analysis at 440 nm was measured.

Antimicrobial activity determination of AgNPs

Agar diffusion method was applied to determine antimicrobial potency of the silver nanoparticles against various cultures of disease causing pathogens including *Candida albicans*, *Candida tropicalls, Candida parapsilosis, Candida glabrata, Candida krusei, Aspergillus flavus, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*. The cultures were obtained from Yeast and Fungal lab, BUITEMS. The disk diffusion method was carried out by the preparation of YM agar plates (Agar 20 g, Glucose 10 g, Malt Extract 3 g, Peptone 5 g and Yeast Extract 3 g dissolved in 1 L and autoclaved at 121°C for 15 min). The agar surface was inoculated with the test pathogens. Then sterile disc were impregnated with 60 µL of silver nanoparticle solution and placed on the surface of the agar incubated at 37°C for 24 h. Diameter of inhibition zone was measured using calliper. Silver nitrate solution impregnated discs were used as controls.

Results

Green synthesis of AgNPs was confirmed by observing gradual change in the filtrate color incubated with aqueous silver nitrate from yellow to light brown after a specific period of incubation. However no change was observed in the controls (containing freshly prepared CD broth with silver nitrate solution). The samples were subjected to UV-visible spectrometry and the obtained spectra depicted absorption bands at 450 nm (Figure 1).

Results obtained from disc diffusion method is given in Table 1 showing the antimicrobial activity of the mycosynthesized silver nanoparticles.

Silver nanoparticles were found to possess enhanced antimicrobial activity against the test pathogens based on their diameter of the inhibition zone

Discussion

Change in color of the fungal filtrate was due to silver ion reduction by the enzyme nitrate reductase as its presence was confirmed by nitrate reductase assay thus resulting in the extracellular synthesis of AgNPs [10]. Similar findings were stated by Ahmad et al. [11] who studied the synthesis of silver nanoparticle in *Fusarium oxysporum* and stated that through the action of enzyme reductase silver nanoparticles were produced [11]. In addition, the process often yields a more steady size distribution pattern due to direct stabilisation of the nanoparticles because of the involvement of the proteins specific for this process [12].

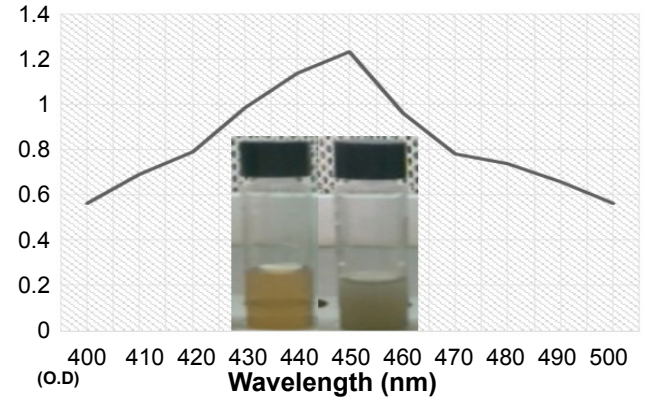

Figure 1: UV-visible spectrum.

S. no.	Test pathogen	Zone of inhibition (mm)
		AgNPs (60 µL)
1	*Aspergillus flavus*	5
2	*Candida albicans*	9
3	*Candida glabrata*	8
4	*Candida krusei*	7
5	*Candida parapsilosis*	6
6	*Candida tropicalis*	8
7	*Escherichia coli*	9
8	*Pseudomonas aeruginosa*	7
9	*Staphylococcus aureus*	9

Table 1: Antimicrobial activity of AgNPs.

However in the absence of enzyme, no colour change was observed in the control flasks as absorption band at 450 nm was not seen which clearly signifies that the enzymatic reduction of nitrate to nitrite contributes in the reduction process for the fabrication of silver nanoparticles [13].

Silver nanoparticles could be employed as antibacterial and antifungal agents based on their antimicrobial activity [14] Silver nanoparticles were found to possess enhanced antimicrobial activity against *Staphylococcus aureus* [15,16], *Escherichia coli* [17,18], *Candida albicans, Candida tropicalis* and *Candida glabrata* with moderate antimicrobial activity against *Pseudomonas aeruginosa* [16], *Candida krusei* and *Candida parapsilosis* and least inhibition was seen in case of *Aspergillus flavus*.

Silver ions are highly reactive as compared to its metallic state which is inert .Silver is easily ionized when it come in contact with moisture present in the fluid therefore silver ions strongly interacts with the microbial cell and not only binds to the tissue proteins causing disruption in the of their nuclear membrane and cell wall [19] but also inhibits their replication by binding to its DNA or RNA, eventually leading to distortion and cell death [20] or may cause disruption in DNA replication by inducing chromosomal aberrations and mitochondrial dysfunction as reported by Chadeau et al. [21].

Conclusion

Extracellular mycofabrication of silver nanoparticles by the fungus *Aspergillus terreus* produces effective nano crystals of silver that possess enhanced anti-microbial spectrum against a variety of pathogenic microbes.

References

1. Gurunathan S, Kalishwaralal K, Vaidyanathan R, Venkataraman D, Pandian SR, et al. (2009) Biosynthesis, purification and characterization of silver nanoparticles using *Escherichia coli*. Colloids and Surfaces B: Biointerfaces 74: 328-335.
2. Gurunathan S, Kalishwaralal K, Vaidyanathan R, Venkataraman D, Pandian SR, et al. (2009) Biosynthesis, purification and characterization of silver nanoparticles using *Escherichia coli*. Colloids and Surfaces B: Biointerfaces 74: 328-335.
3. Sintubin L, De Windt W, Dick J, Mast J, van der Ha D, et al. (2009) Lactic acid bacteria as reducing and capping agent for the fast and efficient production of silver nanoparticles. Appl Microbiol Biotechnol 84: 741-749.
4. Xie Y, Ye R, Liu H (2006) Biosurfactant rhamnolipid preparation of microemulsions. Journal of Colloids and Surface 279: 175-178.
5. Vahabi K, Ali Mansoori G, Karimi S (2011) Biosynthesis of silver nanoparticles

by fungus *Trichoderma reesei* (a route for large scale production of AgNPs). Insci J 1: 65-79.

6. Kowshik M, Ashtaputre S, Kharrazi S, Vogel W, Urban J, et al. (2003) Extracellular synthesis of silver nanoparticles by a silver-tolerant yeast strain MKY3. Nanotechnology 14: 95-100.
7. Mourato A, Gadanho M, Lino AR, Tenreiro R (2011) Biosynthesis of crystalline silver and gold nanoparticles by extremophilic yeasts. Bioinorganic Chemistry and Applications 2011: 546074.
8. Saha S, Sarkar J, Chattopadhyay D, Patra S, Chakraborty A, et al. (2010) Production of silver nanoparticles by a phytopathogenic fungus *Bipolaris nodulosa* and its antimicrobial activity. Dig J Nanomater Bios 5: 887-895.
9. Harley S (1993) Am Biol. Teacher 55: 161.
10. Kashyap PL, Kumar S, Srivastava AK, Sharma AK (2013) Myconanotechnology in agriculture: A perspective. World J Microbiol Biotechnol 29: 191-207.
11. Ahmad A, Mukherjee P, Senapati S, Mandal D, Khan MI, et al. (2003) Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. Colloids Surf B 28: 313-318.
12. Durán N, Marcato PD, Alves OL, de Souza GIH, Esposito E (2005) Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusarium oxysporum* strains. J Nanobiotechnology 3: 1-8.
13. Kumar AS, Ansary AA, Ahmad A, Khan MI (2007) Extracellular biosynthesis of CdSe quantum dots by the fungus *Fusarium oxysporum*. Journal of Biomedical Nanotechnology 3: 190-194.
14. Saravanan M, Nanda A. (2010) Extracellular synthesis of silver bio nanoparticles from *Aspergillus clavatus* and its antimicrobial activity against MRSA and MRSE. Colloids Surf B Biointerfaces 77: 214-218.
15. Afreen RV, Ranganath E (2011) Synthesis of monodispersed silver nanoparticles by *Rhizopus stolonifer* and its antibacterial activity against MDR strains of *Pseudomonas aeruginosa* from burnt patients. Int J Environ Sci 1: 1582-1592.
16. Nithya R, Ragunathan R (2009) Synthesis of silver nanoparticle using *Pleurotus sajor caju* and its antimicrobial study. Dig J Nanomater Bios 4: 623-629.
17. Ray S, Sarkar S, Kundu S (2011) Extracellular biosynthesis of silver nanoparticles using the mycorrhizal mushroom *Tricholoma crassum* (Berk.) Sacc: Its antimicrobial activity against pathogenic bacteria and fungus, including multidrug resistant plant and human bacteria. Dig J Nanomater Bios 6: 1289-1299.
18. Verma VC, Kharwar RN, Gange AC (2010) Biosynthesis of antimicrobial silver nanoparticles by the endophytic fungus *Aspergillus clavatus*. Nanomedicine 5: 33-40.
19. Rajathi K, Sridhar S (2013) Green synthesized silver nanoparticles from the medicinal plant *Wrightia twictoria* and its antimicrobial potential. International Journal of Chemical Technology Research 5: 1707-1713.
20. Costellano JJ, Shafii SM, Ko F, Donate G, Wright TE, et al. (2007) Comparative evaluation of silver-containing antimicrobial dressings and drugs. International Wound Journal 4: 114-122.
21. Chadeau E, Brunon C, Degraeve P, Leonard D, Grossinord C, et al. (2012) Evaluation of antimicrobial activity of a polyhexamethylene biguanide-coated textile by monitoring both bacterial growth and viability (live/dead baclight kit). Food Safety 32: 141-151.