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EVALUATION OF ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICLES BIOSYNTHESIZED FROM PENICILLIUM SPP.

Siddhesh S. Bhagwat, Dr. Arvind S. Kulkarni and Dr. Chanda Parulekar-Berde*

Department of Biotechnology, Gogate-Jogalekar College, Ratnagiri-415612.

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***Correspondence for Author**
Dr. Chanda Parulekar-Berde
Department of Biotechnology, Gogate-Jogalekar College, Ratnagiri-415612.

ABSTRACT

The ability of some microbes such as bacteria and fungi is exploited greatly in order to produce nanoparticles. Fungus like *Neurospora crassa*, *Fusarium oxysporum*, etc were found to be really efficient to produce metallic nanoparticles. Nanoparticles biosynthesis by the microbes is quick, less time consuming and is very cheap as well as cost effective without involving hazardous chemicals. In the present study, *Penicillium* spp. was exploited to biosynthesize silver nanoparticles by reducing silver nitrate. These Ag-NPs were then tested for their antimicrobial activities.

KEYWORDS: nanoparticles, silver, antimicrobial, metallic.

INTRODUCTION

Nanotechnology literally means any technology on a nanoscale that has applications in the real world. Nanotechnology encompasses the production and application of physical, chemical and biological systematic scales ranging from the individual atoms or molecules to submicron dimensions as well as the integration of resulting nanostructures into larger systems.

There have been impressive developments in the field of nanotechnology in the recent past, with numerous methods formulated to synthesise nanoparticles of particular shape and size. There is a growing need to develop environmentally benign nanoparticle synthesis processes that do not use toxic chemicals. As a result, researchers in this field have turned to biological systems for inspiration.[1] Nanoparticles are basically clusters of atoms in the size range of 1-100nm. These metallic nanoparticles have proved to be most effective as it has good antimicrobial efficacy against bacteria, viruses and other eukaryotic micro-organisms.[2,3]

Although nanoparticles synthesised by microbes are very stable, studies have shown that these particles are not mono dispersed and the rates at which they are synthesised are quite slow.[4] The first synthesis of silver nanoparticles by bacteria was reported in 2000. In 2008, biosynthesis of silver nanocrystals by *Bacillus licheniformis* was studied.[5]

After the synthesis, the characterization is very important aspect in order to know the particle size, shape and structure. There are several methods like XRD, SEM, TEM and X-ray crystallography,etc. However, XRD and X-ray scattering studies yielded quantitative characterization of nanostructure and crystalline structure in some nanocomposites.[6]

Silver nanoparticles have unique physical properties. But its most vital application is its role in medical fields for antimicrobial activities against most of the known micro flora as well as anti cancerous properties.[7]

MATERIALS AND METHODS

A. Sample collection

The soil sample was collected from the garden of Gogate-Jogalekar College, Ratnagiri. Soil was collected in sterile petriplates using spatula. It was carried to the laboratory and used for further analysis.

B. Isolation of Penicillium spp.

- **Spread plate method**

Spread plate method was performed to isolate pure culture of Penicillium spp. Successive dilutions were made with sterile saline and plated on potato dextrose agar (PDA) plate. Plates were incubated at room temperature for 48-72 hours.

- **Determination of spore count**

The preparation of fungal spore suspension of the *Penicillium* spp. was carried out by inoculating the spores of *Penicillium* grown on the PDA plate in 10 ml of the sterile saline. 0.1 ml of the suspension was taken and loaded in Haemocytometer chamber to take the spore count of the *Penicillium* spp.

C. Lacto phenol cotton blue (LPCB) staining

The single isolated colony from the plate was scratched, picked up and placed in drop of LPCB stain. After teasing into small bits a coverslip was placed and was observed under 45X microscope (Carl-Zeiss, Germany).

D. Production of fungal biomass

The Potato dextrose broth and Sabouraud's broth were prepared and inoculated with *Penicillium* spp., which was initially isolated. The broth was kept on rotary shaker for 7 days at 110 rpm. The fungal biomass was washed thrice properly and filtered with distilled water using Whatmann filter paper no. 1. To the washed biomass, 200 ml of distilled water was added in the same flasks and kept on rotary shaker for 3 days at 110 rpm. The biomass was filtered with Whatmann filter paper no. 1 and the mycelial biomass was removed. The filtrate and supernatant of the PDB and filtrate of SB were collected. These were labelled as PDB-F, PDB-S and SB-F respectively. These samples were again filtered through Whatmann filter paper no. 1 to remove fungal hyphae and other contaminants. The 1 ml aliquot from each sample was added with 9 ml of aqueous solution of 1mM silver nitrate. It was kept at room temperature for 3 days in dark for the production of silver nanoparticles.

E. Detection and characterization of silver nanoparticles produced by Penicillium Spp.

The preliminary detection of Ag-NPs was carried out by observing colour change of the filtrate which turns to little grey from pale orange.

F. UV-Vis spectrophotometry

The test sample was subjected to optical measurement by UV-Vis spectroscopy between 200-700 nm. The U.V Spectrometer used was CHEMITO-SPECTROSCAN UV2600, India, which is a double beam UV-Vis spectrophotometer.

G. FTIR Spectroscopy analysis

The samples were further subjected to FTIR analysis for more precise detection and characterization of silver nanoparticles generated in samples, and to know the presence of different sorts of functional groups present in the samples. The FTIR used for the analysis of nanoparticles was FTIR/4100-JASCO.

H. Antimicrobial activity

The antimicrobial activity of the silver nanoparticles biosynthesised from Penicillium spp. was checked out using agar cup method of Antimicrobial susceptibility testing. In this method, the two assays were done to check the antimicrobial activity of the silver nanoparticles. The two assays were antibacterial action and antifungal action of silver nanoparticles.

The test cultures used for the assay were *Escherichia coli, Salmonella typhi, Pseudomonas aeroginosa* and *V. cholera*, while the fungal cultures used were *Aspergillus niger, Penicillium* spp. and *Candida albicans*. All the test cultures were procured from Microbial Culture Collection, Department of Microbiology, Gogate Jogalekar College, Ratnagiri.

Mueller Hinton Agar (MHA) plates and Sabouraud's agar plates were prepared for both antibacterial and antifungal assay respectively. Plates were spread uniformly with each bacterial and fungal culture. The wells were made on each plate with sterile cork-borer. 100µl of each sample was added in each well and controls kept for both antibacterial assay and antifungal assay were standard Penicillin antibiotic (40U/2ml) and Clotrimazole (100 U), respectively. These plates were incubated at 37° C for both bacterial and fungal cultures for 24 and 48-72 hours, respectively.

I. Minimum Inhibitory Concentration (MIC)

MIC was determined using increasing concentrations of silver nanoparticles. 5 ml of sterile nutrient broth was added in each tube. Fresh 0.1 ml suspension of each test culture was added aseptically in each respective set of test tubes. The test cultures used were *Escherichia coli, Salmonella typhi, Pseudomonas* spp. and *Cholera* spp. For comparison of results the Negative and Positive control tubes were also prepared.

The tubes were incubated at 37ºC for 24 hrs. After incubation period, according to the turbidity in each set of tubes, the results were compared with the both Positive and Negative controls.

RESULTS AND DISCUSSION

The Penicillium spp. was isolated from the garden soil, on Potato Dextrose Agar (PDA) plate. Further, Lacto Phenol Cotton Blue staining was carried out to confirm the identity of the isolate Penicillium spp. Presence of broom shaped sporangia, confirmed the identity. The fungal culture was maintained on PDA slants and as spore suspension. The biomass was produced in both Potato Dextrose Broth and Sabourauds Broth [Fig 1a,1b].

Fungus like *Neurospora crassa, Fusarium oxysporum, Aspergillus flavus*, are found to be really efficient to produce metallic nanoparticles. Penicillium likewise is also highly capable of synthesizing nanoparticles. *Penicillium* is well known for their antibiotic compounds. As

Penicillium is well known for their antibiotic compounds, the biosynthesis of silver nanoparticles from Penicillium spp. was studied.

Penicillium strain isolated from soil was studied. The colony thus obtained after spread plating was confirmed by LPCB wet mount technique. This culture was inoculated into PDB broth and kept on rotary shaker at 140 rpm for 5 days and the *Penicillium* spp. was found to be capable of producing biomass in 5 days. The biomass was then extensively washed using Whatman'sfilter paper no. 1 with double distilled water. The clean biomass was again taken into flasks containing double distilled water and kept on shaker for 3 days. After 3 days again this was washed as before and into cell free filtrate $AgNO_3$ was added as per its weight in the final volume of the filtrate. The flask was shaken properly and further kept in dark conditions for the appropriate synthesis of the fungus mediated silver nanoparticles.

Fig 1 - Biomass produced in PDB & SB respectively

The samples were used for the synthesis of nanoparticles with the addition of 1mM $AgNO_3$ and was indicated by change in colour from orange to dark grey.[8]

The coloured solutions were centrifuge and the supernatants were scanned for absorption peaks on UV-Vis spectrophotometry. The analysis showed an absorbance peaks at 325.5 nm for both PDB and SB of 0.906 and 0.745, respectively [Fig 2a,2b]. This wavelength is between ideal range for silver nanoparticles.[9] The silver nanoparticles generated from PDB shows higher λ-max than that of the silver nanoparticles generated from SB.

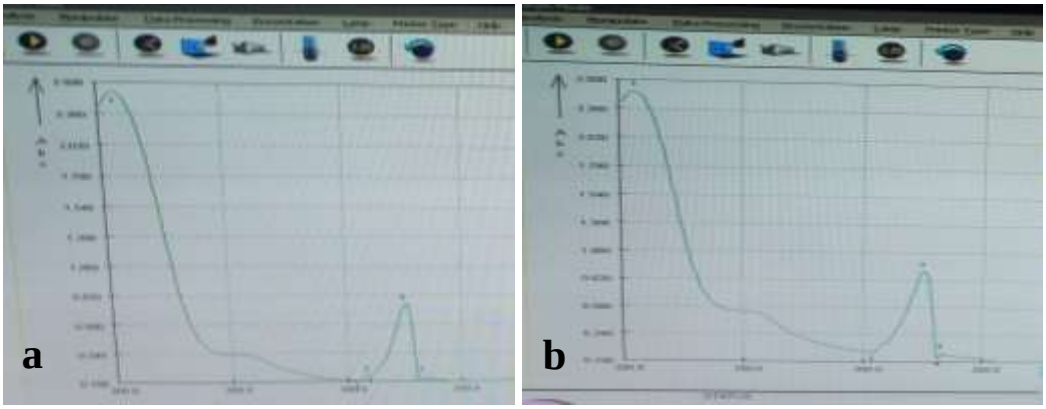

Fig 2- Absorbance of nanoparticles generated by supernatant of PDB & SB

The FTIR analysis showed different peak values ranging from 400cm^{-1} to 4000cm^{-1}. These peaks indicates the presence of various groups in the nanoparticle sample. The FTIR spectrum was used to identify the functional groups of the active components based on the peak value in the region of infrared radiation. The results of the FTIR peak values and functional groups were represented in Fig 3 and 4. FTIR spectrum confirmed the presence of Alcohols, Nitriles, Aromatic compounds, Alkyl halides, Alkenes in both samples. Hence the samples may contain silver nanoparticles which show the antimicrobial activity.

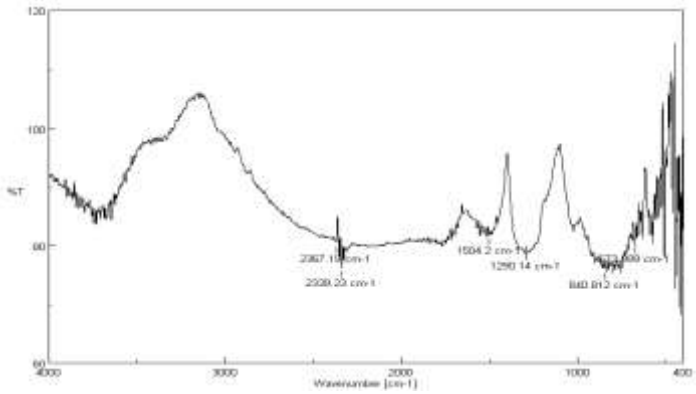

Fig 3. FTIR spectrum of silver nanoparticles generated by PDB

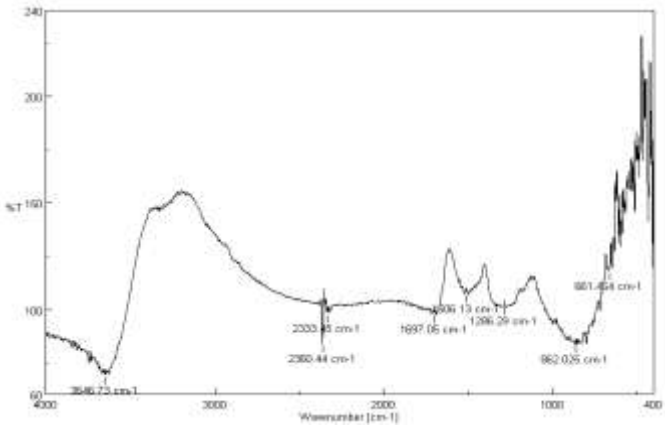

Fig 4- FTIR spectrum of silver nanoparticles generated by SB

Antibacterial and Antifungal activities were seen to be good against *Vibrio cholera*, *Pseudomonas aeruginosa* and against almost all test fungal species In the antibacterial activity, standard penicillin (40U/2ml) was used as positive control. 100µl of the AgNPs solutions of both PDB and SB were used. The diameter of inhibition zones were measured and compared. The activity of these AgNPs was seen in this method along with standard penicillin drug.

After performing the agar cup method against test cultures of *Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa* and *Vibrio cholera*, (Fig. 5, 6) it was found that *Pseudomonas aeruginosa* showed maximum inhibition by all three samples i.e supernatant and filtrate of PDB and filtrate of SB. The zones of inhibition for PDB-S, PDB-F, SB-F were found to be 18, 15 and 17 mm, respectively. While the *E. coli* didn't showed the results for any of the samples as it showed high resistivity against all samples.

The organisms showed zones of inhibition and this confirms that nanoparticles synthesised by fungus are highly toxic to the test pathogenic organisms so the antibacterial activity of silver nanoparticles obeyed the dual action mechanism of antibacterial activity that is the bactericidal effect of Ag+ ions and membrane disrupting effect of the polymer subunit. Hence, formed silver nanoparticles have a great potential use in various fields like medical microbiology, biopharmaceuticals, etc.

Fig.5- Antimicrobial activity of silver nanoparticles solution against P. aeruginosa

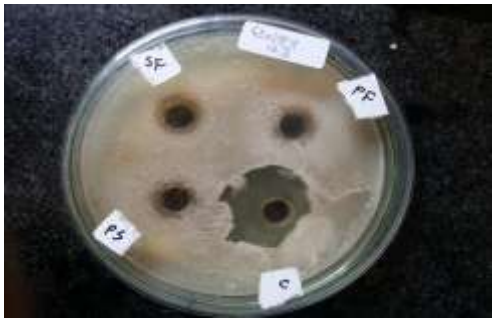

Fig. 6- Antimicrobial activity of silver nanoparticles solution against Vibrio cholera

The antifungal assay was done on the 4 test cultures i.e *C. albicans*, *M. furfur*, *A. niger*, and *Penicillium* spp (Fig. 9). These organisms were cultivated on the sterile SB agar plates. After 24-48 hours of incubation, the cultures showed zone of inhibition except *Penicillium* spp. So, silver nanoparticles were found to be inhibitory to the fungal cultures. *C. albicans* showed zones to PDB-F, SB-F and control of 12, 16 and 16 mm, respectively. *M. furfur* showed 12, 12 and 11 mm zone sizes. *A. niger* showed zones of 14, 17, and 16 mm. While *Penicillium* failed to show inhibition.

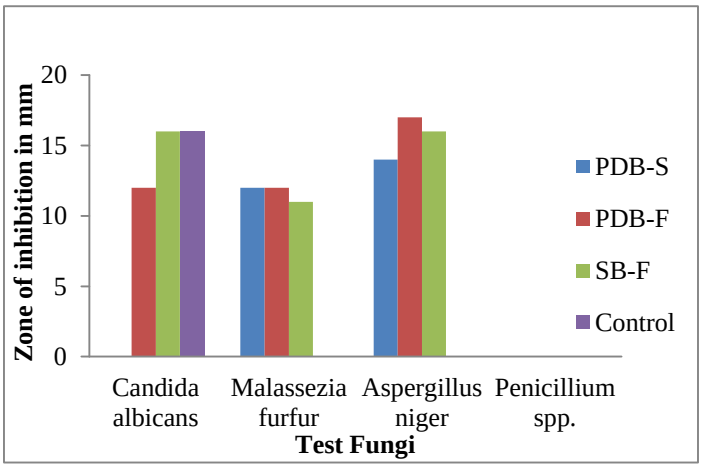

Fig 7- Graphical representation of effects of AgNPs against test fungi

The Minimum Inhibitory Concentration against *S. typhi* and *V.cholerae* was found to be 1000 µg by PDB. While with SB the MIC for *E.coli* was found to be 400 µg.

The antibacterial and antifungal assays were carried out to assess the nature of the silver nanoparticles against various bacteria and fungi which are pathogenic and non-pathogenic as well. *E.coli* and *Vibrio cholerae* used in antibacterial assay are pathogenic to the human health. E.coli is the micro-organism which is generally found in the intestine of the human being, and hence is called as normal human intestine flora. But, when it is not present inside the intestine, it acts as a pathogenic organism. As it is pathogenic it causes various sorts of diseased conditions like diarrhea, continuous illness, damage in intestinal linings and hence stomach-ache. Same way, *V.cholerae* is responsible of causing various diseased conditions like cholera; the water-borne disease, severe watery diarrhea causing dehydration in body.

The silver nanoparticles generated are effective against these pathogenic microbes as they show high antibacterial activity against these pathogenic bacteria. So, these silver nanoparticles can prove enormously effective against the above mentioned infections.

While the Pseudomonas spp. shows highly resistivity against penicillin. Thus, *Pseudomonas* spp.. are mostly involved in carrying out the nosocomial infections in hospitals because most of the strains of *Pseudomonas*. shows high degree of resistivity against common antibiotics which are frequently used on daily basis. It means that the silver nanoparticle samples have more antibacterial activity than that of the standard antibiotic used.

Same way as that of the antibacterial assay the antifungal assay was also carried out and the results were observed. The same method of agar cup was used here to evaluate the antifungal activity of the silver nanoparticles produced from filamentous fungi that is *Penicillium* spp. The test fungal cultures used for this antifungal assay were *Candida albicans, Malassezia furfur, Aspergillus niger* and *Penicillium* spp. itself.

CONCLUSION

Silver nanoparticle producing filamentous fungi was isolated from garden soil using potato dextrose agar. Using Silver nitrate solution, the silver nanoparticles were generated in PDB and SB and was detected by change in colour. Compared to other nanoparticle synthesis methods, this method of biological synthesis seems to be less costly, simpler, eco-friendly and yet more functional and requires less material for the production.[10] The properties of silver nanoparticles can be further administered in various fields like agriculture, medical, industrial and pharmaceutical as well.[11]

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REFERENCES

1. Shankar S., Rai A., Ahmad A. and Sastry M., Rapid synthesis of Au, Ag and metallic bimetallic Au core and Ag shell nanoparticles using Neem leaf broth, J. Colloid Interface Sci., 2004; 275: 496-502.
2. Gong P., Li H., Wang K., Zhang S., Yang X., Preparation and antibacterial activity of Fe_3O_4@Ag nanoparticles, Nanotechnology, 2007; 18: 604-611.
3. Rai M., Yadav A., Gade A., Silver nanoparticles as a new generation of antimicrobials, Biotechnol. Adv., 2009; 27: 76-83.
4. Philip, D. Biosynthesis of Au, Ag and Au-Ag nanoparticles using edible mushroom extract, Spectrochemica Acta Part A, 2009; 73: 374-381,

5. Kalimuthu K., Pandian R., Babu S., Venkataraman D., Bilal M., Biosynthesis of silver nanocrystals by Bacillus licheniformis, Colloids and Surfaces B: Biointerfaces, 2008; 65:150-153.
6. Depan, P. Kumar, R. Singh P., Cell proliferation and controlled drug release studies of nanohybrids based on chitosan-g-lactic acid and montmorillonite, Acta Biomaterialia, 2009; 5: 93-100.
7. Verma S., Abirami S. and Mahalakshmi V., Anticancer and antibacterial activity of silver nanoparticles and its synergestic effect with antibiotics, J. Microbiology and Biotechnology Research, 2013; 3: 54-71.
8. Vaibhabi K., Mansoori G., Karimi S., Biosynthesis of silver nanoparticles by fungus Trichodermareesei, Indian Science Journal, 2011; 1: 65-69.
9. www.researchgate.net
10. Khouloud M. M, Abou-El-Nour, Abdulrahman Al-Warthan, Synthesis and applications of silver nanoparticles, Arabian Journal of Chemistry, 2010; 3: 135-140.
11. Mandal D., Bolander M., Mukherjee P., Mukhopadhyay D., Sarkar G., The use of microorganisms for the formation of metal nanoparticles and their applications, J. Applied Microbiology & Biotechnology, 2006; 69: 485-492.