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Evaluation of antimicrobial activity of biologically synthesized silver nanoparticles from filamentous fungi

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Abstract: In this paper we have reported the biosynthesis of silver nanoparticles using *Penicillium sp.* was isolated from the soil sample collected from Pallavakam beach of Chennai, Tamil Nadu India. When the fungal biomass was challenged with 1mM Silver nitrate (AgNO3) results in the change of solution color in to dark brown shows the formation of Silver nanoparticles(SNP).These Silver nanoparticles were characterized by UV-Vis spectrophotometric analysis which shows the absorption peak at 419nm confirms presence and formationof silver nanoparticle. These biosynthesized SNP were further characterized by X-ray diffraction spectroscopy (XRD) which determine the metallic nature of the SNP and Field Emission Electron Microscopy (FESEM) which shows the silver nanoparticles are spherical,well dispersed and particle particle size is around 20-45nm. These silver nanoparticles showed good antibacterial activity against various bacterial pathogens and also enhances the antibacterial activity of Amoxicillin were studied.
Key word: *Penicillium sp.*, Silver nanoparticles, FESEM, UV-vis spectrophotometer, XRD.

Introduction

Nanotechnology deals with the technology at nanolevel which have emense application in the field of biotechnology, agriculture, biomedical,drug delivery etc. During recent years resistance acquired by the pathogenic micro-organisms to various available antibiotics in the market has become a serious health issue[1,2]So there is an urgent demand to find out new bactericidal material, which counter act against bacterial resistance among them silver, silver ions and silver based compounds has been used from very ancient times as an antibacterial material to fight with various infections and its antibacterial , antiviral property and antioxidant against neurological diseases like Alzheimer's and some cancers have also been studied.[3-8]

Different organisms have been used for the biosynthesis of nanoparticles like plants, bacteria, yeast and fungi [9,10,11] but fungi has advantage over other organisms like simple to handle, eco-friendly, requires very less time, produces the large amount of extracellular enzymes and can uptake the metal ion as compared to physical and chemical method .

In this paper we have reported the biosynthesis of silver nanoparticles using filamentous fungi Penicillium sp. and the formation of silver nanoparticles were characterized by UV-vis spectrophotometer, XRD and FESEM. After the formation of silver nanoparticles which have been checked for its antibacterial activity against various pathogens and evaluate its synergistic effect with Amoxicillin were studied.

Materials and Methods

Sample collection

Soil sample was collected from Pallavakkam beach of Chennai Tamil Nadu India. Soil Sample was collected from 2 to 3cm depth with the help of sterile spatula. Samples were transferred in to sterile plastic bags and brought to the Biomedical and Research laboratory and stored in a refrigerator at 4^0c up to further process.

Isolation of fungal culture

Isolation of soil fungi was performed by serial dilution and spread plate method. One gram of soil sample was serially diluted in sterilized distilled water to get the concentration which ranges from10^{-1} to 10^{-6}. A volume of 0.1 ml of each dilution was transferred aseptically to SDA plates. The plates were incubated at room temperature for 5 days. The fungal isolates were sub cultured on SDA plates in order to segregate the isolated fungi in to pure culture. Pure isolated fungal culture was maintained at 4^oc for further studies[12]

Microscopic and colony characterization

Penicillium sp. was observed by the author expertise using hand lens and the colony morphology was recorded with respect to color, shape, size and nature of colony and also by laboratory manuals in the department of Biomedical Engineering Sathyabama University Chennai.

Biosynthesis of silver Nanoparticles

Penicillium sp. was used for the extracellular biosynthesis of silver nanoparticles. Fungal biomass was grown aerobically in a liquid medium containing (g/L): KH_2PO_4 7.0; 2.0 K_2HPO_4 $MgSO_4$. $7H_2O$ 0.1; $(NH_4)2SO_4$ 1.0; yeast extract 0.6; glucose 10.0 at 25 ± 3^0c. After incubation, the biomass was filtered using Whatman filter paper No.1 and extensively washed two thee times with distilled water to remove residual parts. The fresh and clean biomass was taken into an Erlenmeyer flask, containing 100ml of deionized Milli-Q water. The flask was incubated at 25^Oc in a shaker incubator at 140 rpm for 72 hours. The biomass was filtered again with Whatmann filter paper No.1 and the cell free extract was used further. 1mM $AgNO_3$ was prepared and 50ml was added to the cell-free extract and kept further in the incubator at 25^Oc, 140rpm for 72hours in dark condition. .

Characterization of Silver Nanoparticles

After the incubation for 72 hours the solution color changes in to dark brown and the absorbance of the reaction synthesized mixture was measured by using UV-visible spectrophotometer between 300-700nm analysis. These biologically synthesized silver nanoparticles were dried and powder form of sample was subjected for XRD analysis. After these silver nanoparticles has been further characterized by FESEM. For this sample has been prepared by centrifugation of the solution at 13,000 rpm for 15minutes.After that supernatant was discarded and pellet was dissolved in acetone, dried it in the petri plate for some time in to powder form and subjected to FESEM analysis.

Evaluation of Antibacterial activity

Biologically synthesized SNP from *Penicillium sp.* were checked for its antimicrobial activity against *Staphylococcus aureu, Escherichia coli, Vibrio cholera Bacillus cereus* and *Proteus vulgaris* by disc diffusion method. The combined effect of SNP with antibiotic Amoxicillin were used to find out synergistic effect against above bacterial pathogens. The Zone of inhibition can be measured after 24 hours at 37^0c.

Results and Discussion

Penicillium sp. (Fig 1) has been used for the biosynthesis of silver nanoparticles. When the fungal biomass was challenged with 1mM silver nitrate, change of the solution color in to dark brown indicates the formation of silver nanoparticles (Fig 2).

Fig 1 : *Penicillium Sp.* on SDA plate

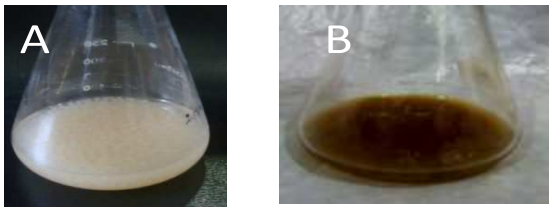

Fig 2: synthesis of Silver nanoparticles from *Penicillium Sp*.
(A) Before addition $AgNO_3$
(B) After addition of $AgNO_{3.}$Color change

These nanoparticle were further characterized by UV-vis spectrophotometric analysis which shows the absorption peak at 419nm due to Plasmon resonance which indicates the synthesis of silver nanoparticles and absorption peak is specific for the silver nanoparticles (Fig 3)[13,14,15].

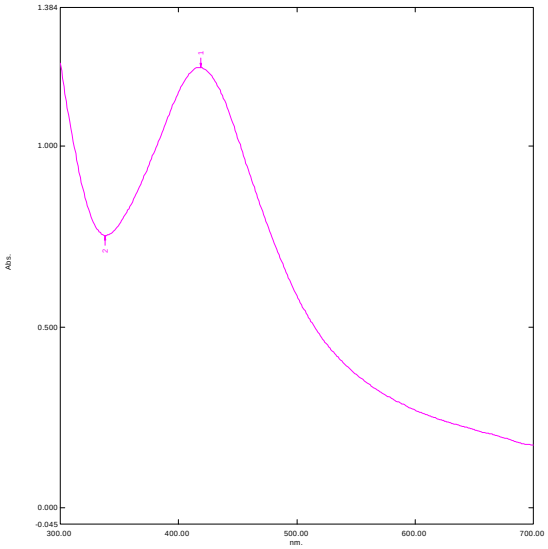

Fig 3: Detection of Biologicallgy synthesized Silver nanoparticles by UV–Vis spectrum

These biologically synthesized silver nanoparfticles from Penicillium Sp. were further characterized byX-ray diffraction analysis which shows some intense peaks which determines the metallic nature of the silver nanopaparticles, nanocrystallites which confirms the formation of silver nanoparticles and diffraction peaks which confirms the synthesis of Silver nanoparticles were theta value at 38^{0} [16] Fig 4**.**

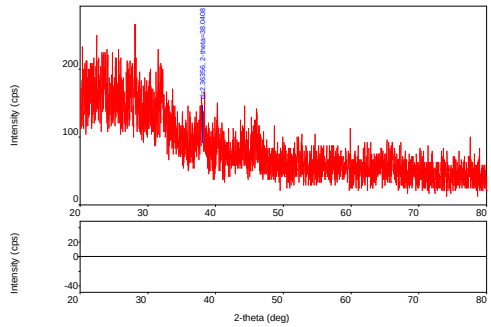

Fig 4: XRD pattern shows silver nanoparticles synthesized from *Penicillium Sp.*

These biologically synthesized silver.nanoparticles using *Penicillium sp*. were further characterized by FESEM which showed the surface topology of nanoparticles,uniform in diameter and are spherical well dispersed and particle size is around 20 -45nm Fig 5**.**

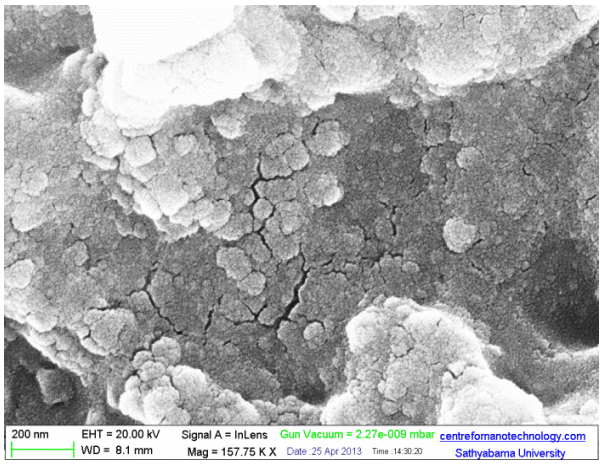

Fig 5: FESEM image of Silver nanoparticles syntheiszed from Penicillium sp.

The antibacterial activity of silver nanoparticles were carried through disc diffusion method against different bacterial pathogens viz., *Staphylococcus aureus, Bacillus cereus, E. coli, Vibrio cholerae* and *Proteus vulgaris*.. Each disc has been impregnated with 25µl silver nanoparticle solution. The synergistic effect of silver nanoparticles with commercially available antibiotic Amoxicillin (10mcg disc) was also analyzed aong with this. The highest antibacterial effect was shown against *Bacillus cereus* (26mm), *E. coli* (25mm), *Proteus vulgaris* (19mm),*Staphylococcus aureus* (14mm) and *Vibrio cholerae* (13mm) The results showed that the these silver nanoparticles showed good antibacterial activity and also synergistic effect of Amoxicillin in presence of SNP was increased remarkably . (Fig 6) shows the graphical representation of the combined effect of silver nanoparticles with Amoxicillin antibiotic. The synergistic activity of silver nanoparticles enhanced the antibacterial property of Amoxicillin studied during the study period.

Table 1: Zone of inhibition (mm) of Amoxicillin against test pathogens in the presence and absence of silver nanoparticles.

Sl. No.	Pathogenic Bacteria	Fungal fiterrate	Ag NPs 25µl/disc	Amoxicillin (10mcg disc)	Amoxicillin+ AgNPs
1	*S. aureus*	8	10	11	14
2	*Bacillus cereus*	9	13	15	26
3	*E. coli*	8	12	16	25
4	*Vibrio cholera*	7	9	8	13
5	*Proteus vulgaris*	10	12	13	19

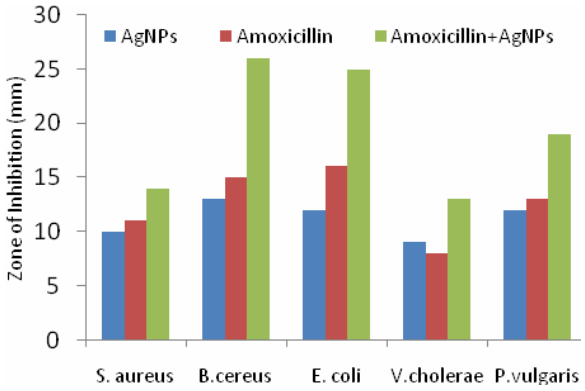

Fig 6: Graphical representation shows the synergistic effect of Amoxicillin with AgNPs against Selected bacterial pathogens

Conclusion

Biosynthesis of silver nanoparticles using *Penicillium sp.* is quite simple, cheap, stable and environmentally friendly. Further these biologically synthesized silver nanoparticles alone and in combination with antibiotic Amoxicillin can be used as an effective bactericidal material against human bacterial pathogens.

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