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Research Article

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Facile and green synthesis of silver nanoparticles from *Penicillium italicum* and its antimicrobial property in combination with Sparfloxacin

[1]Shahnaz Majeed*, [1]Mohd Syafiq bin Abdullah, [1]Gouri Kumar Dash and [2]Anima Nanda

[1]Faculty of Pharmacy and Health Science, Universiti of Kuala Lumpur Royal College of Medicine, Tasac Ipoh Perak, Malaysia
[2]Faculty of Bioscience and Chemical Engineering, Sathyabama University, Chennai, TamilNadu, India

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ABSTRACT

Now a days Nanobiotechnology is the promising area of research which controls the materials at nano level, having tremendous application in the area of medicine. Nanoparticles contain remarkable antimicrobial property to overcome the bacterial resistance developed by the pathogenic bacteria to varied amounts of antibiotic drugs. Silver nanoparticles have advantage over other metallic nanoparticles as it posses broad range of bactericidal property. Here in this paper we have reported the extracellular biosynthesis of silver nanoparticles from Penicillium italicum isolated from the soil collected from the campus of Sathyabama University Chennai Tamil Nadu India. After this nanoparticles were characterized by various techniques followed by antibacterial activity in combination with Sparfloxacin. Formation of dark brown in conical flask to cell free extract indicates the formation of silver nanoparticles. Synthesis by addition of 1Mm $AgNO_3$.UV vis spectrophotometer showed the absorption peak at 418nm indicates the formation of nanoparticles. Atomic Force Microscopy (AFM) showed that nanoparticles are spherical and size is around 40nm.Nanoparticles showed good antibacterial alone and enhances the bactericidal activity of Sparfloxacin studied during the present study.

Key words: Silver nanoparticles, AFM, UV-Vis spectrophotometer.

INTRODUCTION

Nanotechnology deals with the technology which is at atomic or molecular level with the synthesis, characterization and application of nanoparticles whose size ranges from 1-100nm.Nanoparticles can be determined based upon shape, size and structure [1]. Nanoparticles contain large amount of surface atoms as compared to micro particles so that most portion of nanoparticles faces in direct contact with the outside environment which makes them unique by as compared to another by improving their functional capability. Now a day's these metallic nanoparticles showed very good applicability in various fields like healthcare, electronic, biosensing, agroforestry catalysis and agriculture [2,3,4,5]. From past so many decades in ancient era it has been known that silver posses antibacterial activity and hence it diverts the attention for the most of the researchers to work on the silver nanoparticles(Ag-NPs) which posses very good antimicrobial and anti inflammatory property hence used for topical ointment and wound healing, [6,7,8]etc.

Synthesis of nanoparticles by using biological method using living organism as nanofactories is quick, clean, cost-effective, biocompatible and environmentally friendly [9,10] than chemical and physical method[11]. Different biological microorganism as a nanofactories have been used for the synthesis of nanoparticles like plants [12]

bacteria [13] and fungi [14]. Fungi have advantage over other biological organisms as it is highly tolerant toward the uptake of metal ions and produce large amount of extracellular enzymes [15]. Several living organisms have been used for the synthesis of Ag-NPs as nanofactories either intracellularly or extracellularly by using ecofriendly nanofactories [16, 17]. Ahmad et al exploited fungi *Fusarium oxyporium* for the biosynthesis of silver nanoparticles and particles size ranges from 5 to 15nm[18] and there are also several available reports now days which showed the synthesis of silver nanoparticles using fungi like *Fusaium solani*[19], *Penicillium fellutanum*[14], *Cryphonectria sp*.[20], *Phomaglo merata*[21] *Alternaria alternate*[22],] etc.

Ouraim and objective of the present study is to biosynthesize Ag-NPs by using *Penicillium italicum* isolated from the campus Sathyabama university Chennai Tamil nadu India followed by characterization of these nanoparticle by UV-Vis spectroscopy, AFM analysis and to evaluate the antibacterial activity of these Ag-NPs alone and its synergistic effect with commercial available antibiotic Sparfloxacin against various gram positive and gram negative bacteria viz., *Staphylococcus aureus, Bacillus cereus, E. coli,* and *Proteus vulgaris.*

EXPERIMENTAL SECTION

Sample collection

Soil sample was collected from the campus of Sathyabama university Chennai Tamil Nadu India. Sample of soil was collected from the depth 3 to 4cm using sterile spatula. Soil sample was then transferred in to sterile plastic bags and brought to the Biomedical and Research laboratory Microbiology section and stored in a refrigerator at 4^0c up to further process.

Isolation of fungal culture

The fungi in soil has been isolated by performing the serial dilution and spread plate method. Air dried one gram of soil was serially mix with sterile water and than dilute it to get the concentration which ranges from10^{-1} to 10^{-6}. 0.1 ml volume of solution of each dilution was than aseptically transferred on PDA plates. After that using sterile glass rod to distribute uniformly on the PDA plates. These PDA plates were incubated at room temperature for 3 -5 days. After the appearance of fungal strains on the PDA plates The fungal strains were isolated and cultured them again on PDA plates, so that pure culture has to be isolated. Pure isolated fungal culture was maintained at 4^oc for further studies.

Microscopic and colony characterization

Penicillium italicum isolated was observed by using microscope and the colony morphology was observed with respect to color, shape, size and nature of colony and also by using laboratory manuals available in the department of Biomedical Engineering Sathyabama University Chennai.

Synthesis of silver Nanoparticles

Penicillium italicum was exploited for the extracellular biosynthesis of silver nanoparticles. *Penicillium italicum* fungi 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 to three times with Milli-Q water in order to remove the media. The fresh and clean biomass was taken into an flask, containing 100ml of deionized Milli-Q water. The flask was kept in a shaker for three days at 140 rmp at 25^oc. The fresh biomass was filtered again using Whatmann filter paper No.1 and the cell free extract was used further studies. 1mM $AgNO_3$ was added to the cell-free extract and kept in a shaker for two days.

Characterization of Silver Nanoparticles

After addition of$AgNO_3$ the solution was incubated for 24 to 48hours the change in solution color and the absorbance of the reaction mixture was analysed by using UV-visible spectrophotometer between 300-600nm analysis. After the synthesis nanoparticles were characterized by using microscopic technique AFM used to check the particle size and agglomeration. Two dimensional and three dimensional image of AFM were taken which are used to check average particle size, and roughness of nanoparticles. For AFM an anlysis the sample was prepared by sonicate it for 5 minutes followed by centrifugation at 15000rpm then prepare thin film of the pellet on glass slide and subjected for AFM analysis.

Antimicrobial assay

Antimicrobial assay of silver nanoparticles was carried out against gram positive and gram negative pathogenic bacteria such as *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Vibrio cholera* and *Proteus vulgaris* by disc diffusion assay.[29] The combined effect of Ag-NPs with Sparfloxacin was evaluated to find out the synergism against the above pathogens. zone of inhibition was measured after overnight incubation at 37^0c. The experiment has been repeated three times and average mean has been calculated by using standard deviation.

RESULTS AND DISCUSSION

The change in color of the solution in to dark brown was observed after the addition of $AgNO_3$ to the cell filtrate of *Penicillium italicum* indicates the formation of Ag-NPs due to the surface Plasmon resonance effect and reduction of Ag^+ to $Ag^{0.}$ (Fig1)[23].The color of the solution retained even after the incubation for 72 hours which indicates that the nanoparticles are well dispersed and there was no aggregation occurs within these nanoparticles. Many researchers shows same results in the past [24,25,26].These silver nanoparticles were further characterized by spectrophotometric analysis (Fig 2).The UV-Vis spectra showed the strong absorption peak at about 418nm indicated the silver nanoparticles were uniform in size, well dispersed and stable.[27].

AFM analysis used to determine the size, agglomeration, surface roughness of nanoparticles. Three dimensional images of AFM showed the particle height and also informed about the average roughness of the silver nanoparticles (Fig 3) while as two dimensional image of AFM showed the particle size (Fig 4). From two dimensional image of AFM showed that the particle size was around 40 nm, spherical and polydispersed. Three dimensional image of AFM showed that average size is quite under 40nm.

In vitro antibacterial analysis of silver nanoparticles synthesized from *Penicillium Italicum* were analyzed through disc diffusion method was carried out, without antibiotic and in combination with the c antibiotic Sparfloxacin (5mcgdisc) against different pathogenic microorganism viz., *Staphylococcus aureus, Bacillus cereus, E. coli, Vibrio cholera* and *Proteus vulgaris*. Each disc has been impregnated with a concentration solution of Ag-NPs 20µg on all plates and also cell free filtrate was used as negative control. Ag-NPs in combination with Sparfloxacin showed maximum activity against *S. aureus* (29 mm), *B. cereus* (28 mm), *V. cholerae* (26 mm), *E. coli* (25 mm), followed by *P. vulgaris* (24mm), as showed in Table1. (Fig 5) shows the graphical representation of the combined effect of Sparfloxacin and Ag-NPs against the above pathogens. The results showed that the Silver nanoparticles alone and in combination with sparfloxacin enhance the antimicrobial activity of Sparfloxacin in a combined formulation studied during the study period.

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

SI No.	Pathogenic Bacteria	Fungal Filtrate	AgNPs (40µg/disc)	Sparfloxacin 5mcg disc	Sparfloxacin + AgNPs
1	*S.aureus*	9 ± 0.02	21± 0.55	24 ±1.60	29±0.87
2	*Proteus vulgaris*	10 ± 0.30	22 ± 0.40	22 ±0.69	24±1.88
3	*Bacillus cereus*	8± 0.70	20 ± 1.70	22 ±1.80	28±0.58
4	*Vibrocholerae*	9 ± 0.04	18 ± 1.52	21 ± 0.90	26±0.72
5	*E.coli*	9 ± 0.90	20 ± 0.80	21± 1.70	25±1.39

Fig 1: Synthesis of silver nanoparticles from *Penicillium Italicum*. (A) Before addition of $AgNO_3$ (B) After addition of $AgNO_3$,color change

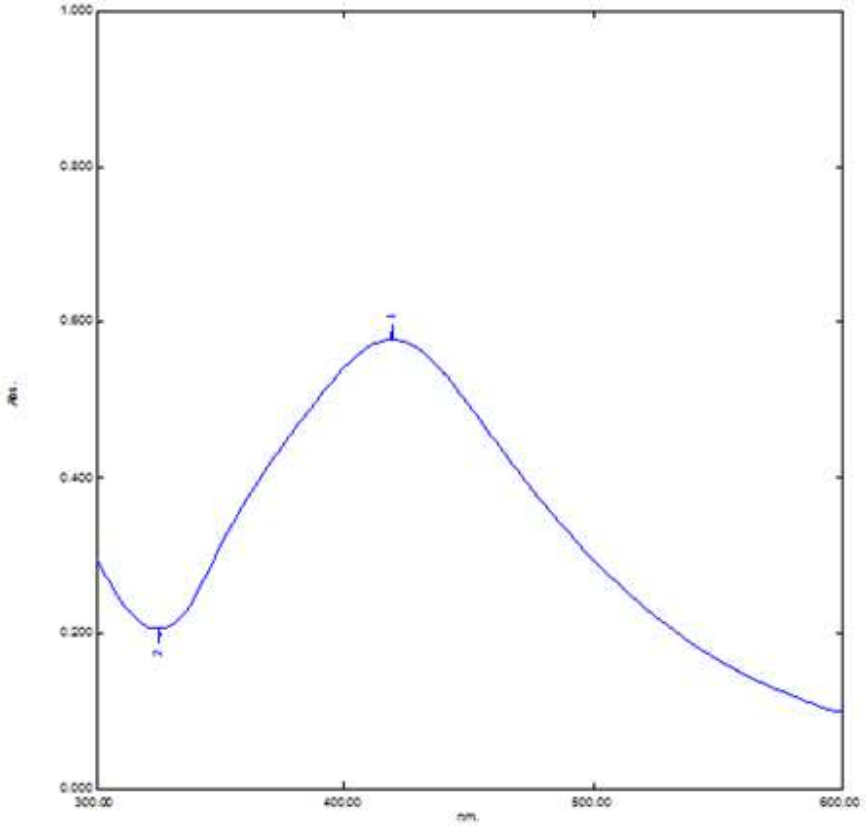

Figure 2: UV–vis spectra recorded silver nanoparticles synthesized from *Penicillium italicum*

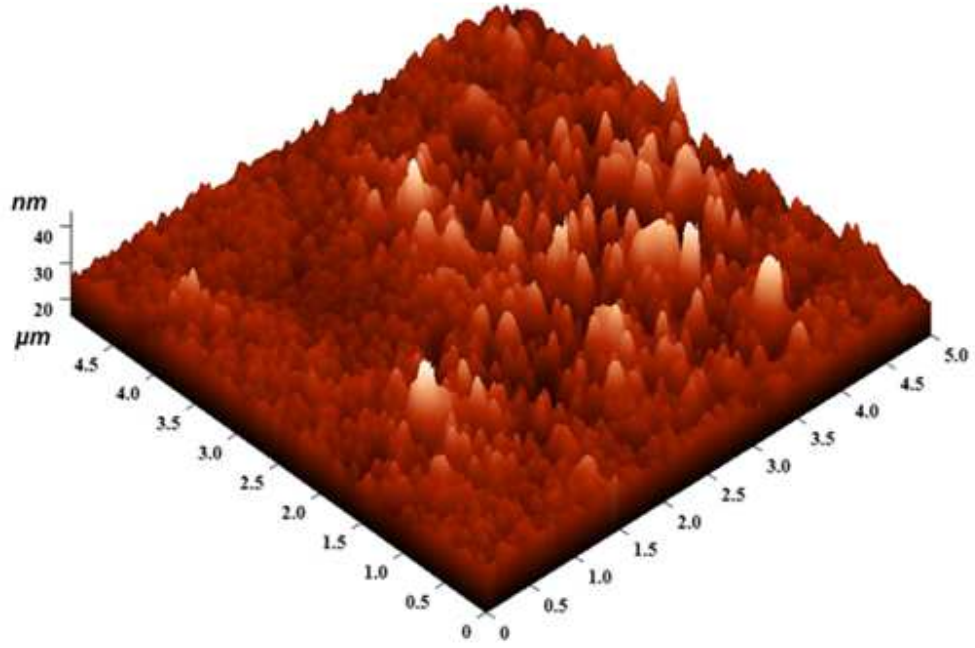

Fig 4: 3D picture of Atomic Force microscopy (AFM) shows the particle height, roughness and inhomogenity of cluster formation of silver nanoparticles synthesized from *Penicilium italicum*

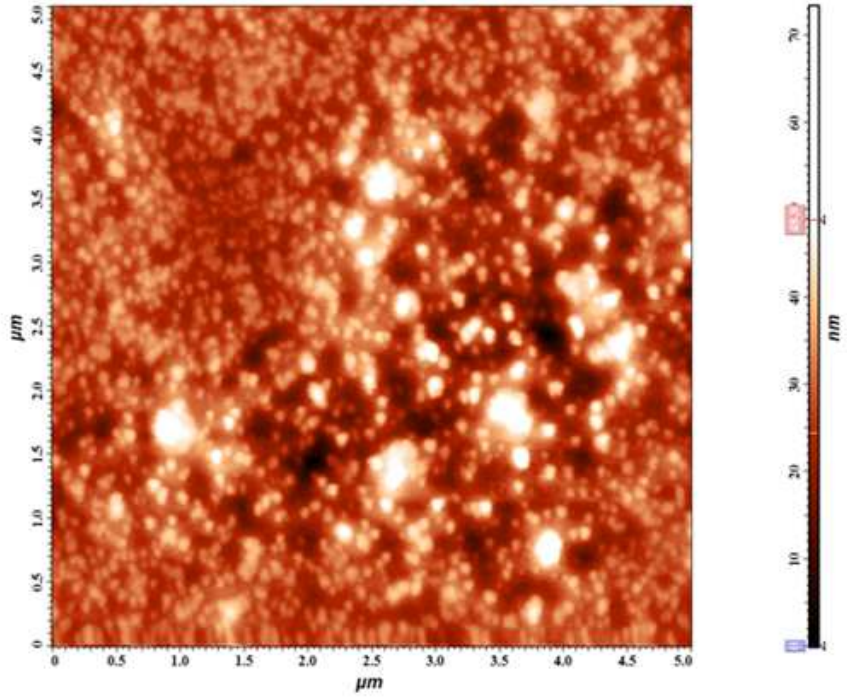

Fig 5: 2D picture of Atomic Force Microscopy (AFM) of silver nanoparticles synthesized from *Penicillium italicum*. Particle size around 40nm

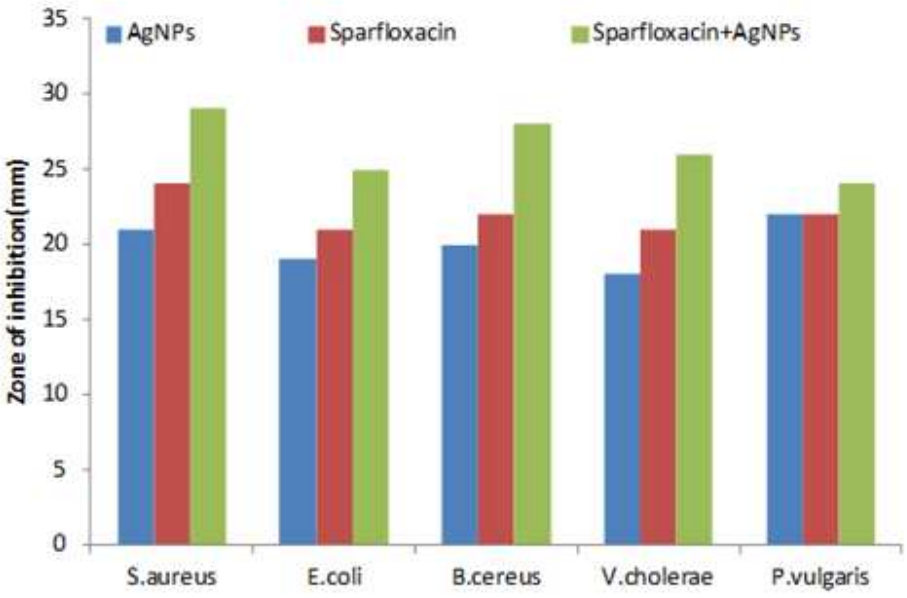

Fig (6): Graphical representation of the combination Sparfloxacin with Ag-NPs against the selected pathogens.

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