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Research Article

IMPROVED BACTERICIDAL PROPERTY OF SILVER NANOPARTICLES FROM PENICILLIUM PINOPHILUM (MTCC 2192) IN A COMBINED FORM WITH CARBICILLIN AND MOXIFLOXACIN

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ABSTRACT

Objective: In the recent scenario, progress in nano-biotechnology research has made a great development particularly in the field of medicine based on metallic nanoparticles with antibacterial property to combat the pathogenic bacteria, who are resistance to varied antibiotics. Silver nanoparticle has its own advantages in order to kill the microbes effectively.

Methods: In this paper, extracellular biological synthesis of silver nanoparticles was made from *Penicillium pinophilum* (MTCC 2192). The characterization of the nanoparticles was carried out as well as its antibiotic efficacy was evaluated in addition with the antibiotics.

Results: The development of yellowish brown color in the conical flask suggested the formation of silver nanoparticles (AgNPs). The AgNPs was investigated by the UV-Vis spectroscopy, which confirmed the presence of silver nanoparticles in the prepared solution. Characters of these silver nanoparticles were further studied by Fourier Transform Infrared (FTIR) Spectroscopy, Field emission scanning electron microscopy (FESEM) and Atomic force microscopy (AFM) analysis. The particle sizes were recorded within 33.29 to 66.97nm and the roughness of the silver nanoparticles were noted. The synthesis process was found quite fast, convenient, ecofriendly and cost effective. The nanoparticles produced during the study period were found to have wider antibacterial property and also it showed the enhanced efficacy in combination with carbicillin and moxifloxacin against selected bacterial pathogens.

Conclusion: The synergistic mode of antibiosis in between nanoparticles synthesized from *P. pinophilum* (MTCC 2192) and carbicillin & moxifloxacin was found to be more effective against pathogenic bacteria during the present study.

Keywords: AgNPs, *P. pinophilum* (MTCC 2192), AFM, FESEM, FTIR, UV-Vis Spectrophotometer.

INTRODUCTION

Studies on nanotechnology, especially silver nanoparticles have wide range of applications in the healthcare sectors, agriculture, ceramics, staining pigments and environment etc. From ancient times, silver has been known for its disinfectant property and were in the use of various traditional medicines as well as antimicrobial drugs [1]. It has been found that silver is safe in low concentration for human cells but lethal for bacteria and viruses [2].

The antimicrobial property of silver nanoparticles and its related compounds are studied by earlier workers [3, 4] and it is found that they also posses anti inflammatory, antiviral, anti-platelet and antifungal activity [5, 6]. Recent studies on the metallic nanoparticles, silver have good antimicrobial activities [7] and its combined effect with antibiotics has also been reported by different workers [8]. It has been used in many ways, formulation of ointments to prevent infection in burns and wounds [9, 10, 11]. Silver nanoparticles are used to synthesize by physical, chemical and biological methods [12], but physical and chemical methods are costly, time consuming and toxic [13].

Synthesis of nanoparticles by biological way is wiser in the sense of cost effective, convenient and free from any chemical [11, 14, 15, 16]. The ability of the fungi and bacteria in order to synthesize nanoparticles in a controlled manner to form new materials is test worthy [17]. Development of different protocols in the production of nanoparticles utilizing fungi [18, 19] and bacteria [20, 21] is in a full swing. The aim and objectives of the present study is to biosynthesize silver nanoparticles from *Penicillium pinophilum* MTCC (2192) procured from IMTECH, Chandigarh, India by extracellular method, to confirm the formation of silver nanoparticles by UV-Vis spectroscopy followed by various microscopic characterization and to evaluate its (silver nanoparticles) efficacy as a bactericide as well as its synergistic effect with carbicillin and moxifloxacin in order to combat the growth of selected bacterial pathogens viz., *Staphylococcus aureus, Bacillus cereus, E. coli, Vibrio cholera* and *Proteus vulgaris.*

MATERIALS AND METHODS

The culture of *Penicillium pinophilum* (MTCC-2192) strain was obtained from the Institute of Microbial Technology (IMTECH) Chandigarh, India. The culture was maintained in Sabouraud Dextrose Agar (SDA) medium and sub cultured from time to time to optimize its viability and virulence in the laboratory during the present study.

Synthesis of silver nanoparticles

Penicillium pinophilum (MTCC- 2192) was utilized 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^{0}c. After incubation, the biomass was filtered using Whatman filter paper No.1 and extensively washed 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^{0}c 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^{0}c, 140rpm for 72hours in dark condition.

Characterization of silver nanoparticles

The samples were observed for alteration of solution color and maximum absorbance was analyzed using UV- spectrophotometer. 1ml of sample supernatant was taken after 24hours and absorbance was measured by using UV-visible spectrophotometer between 300-600nm. The sample was subjected to FTIR spectroscopy analysis. Two milligram of the sample was taken and pressed to form the pellet. The sample was kept into the sample holder and FTIR graph were taken. After the synthesis, the silver nanoparticles were further characterized by AFM which was used to determine the particle size and agglomeration of the nanoparticles. The two dimensional and three dimensional image of AFM were taken which showed the particle height and average roughness of silver

nanoparticles. The sample used for the analysis was sonicated for 5 minutes, centrifuged and made into a thin film for AFM analysis. FESEM was used to determine the surface morphology and size of the nanoparticles, for which sample was prepared by centrifugation, dried into powder form and subjected to SEM analysis.

Antibacterial analysis

The silver nanoparticles were checked for its antibacterial activity by disc diffusion method [22, 23]. The antimicrobial activity of the prepared silver nanoparticles from *Penicillium pinophilum* (MTCC 2192) was tested against the pathogenic bacteria such as *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Vibrio cholera* and *Proteus vulgaris*. The combined formulation of silver nanoparticles with standard antibiotic discs of Carbicillin and Moxifloxacin were used to find out the synergistic effect against the above pathogens.

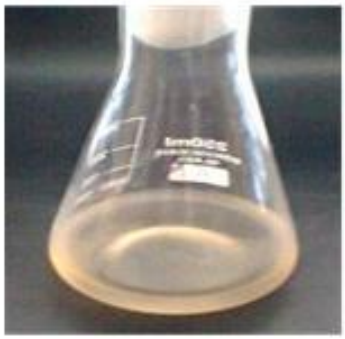
The zone of inhibition was measured after overnight incubation at 37^0c.

Calculation for Increase in fold area

The mean of increase in fold area were calculated by the mean surface area for the zone of inhibition of each antibiotics that were used alone and antibiotic + AgNPs separately. The increase in fold area of different pathogens for antibiotics and antibiotics + AgNPs was calculated by using this equation: $(B^2 - A^2)/A^2$, where A was the antibiotic alone and B was the antibiotic + AgNPs respectively [8, 22].

RESULTS AND DISCUSSION

The biomass of *Penicillium pinophilum* (MTCC 2192) was used for the synthesis of silver nanoparticles. After the treatment of silver nitrate, the color of the solution changed into brown indicated the formation of silver nanoparticles (Fig 1) [24].



(A)

(B)

Fig. 1: Biosynthesis of Silver Nanoparticles - change of colour in the reaction (A) Before addition of $AgNO_3$, (B) After addition of $AgNO_3$.

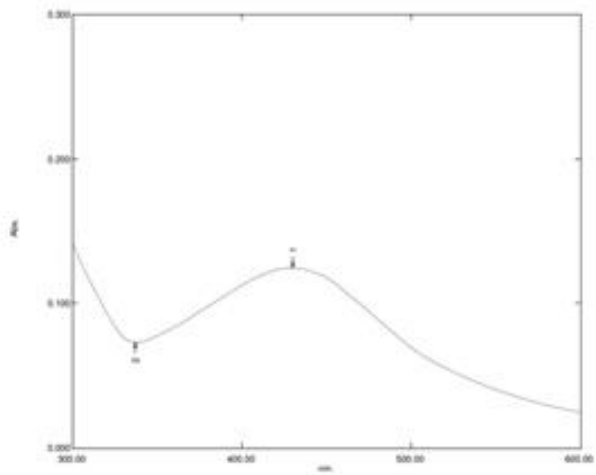


Fig. 2: UV-Vis spectrum of fungal filtrate containing silver nanoparticles synthesized from *P. pinophilum*(MTCC-2192)

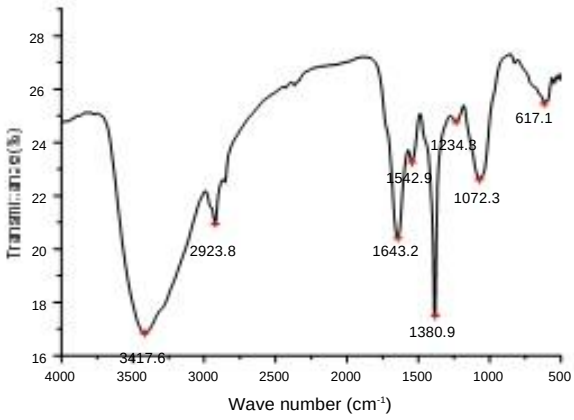


Fig. 3: FTIR analysis recorded of Silver Nanoparticles synthesized from *P. pinophilum(MTCC2192)*

The silver nanoparticles were confirmed by UV-visible spectrophotometric analysis (Fig 2). The UV-Vis spectra showed the absorption band peak at about 425nm indicated the silver nanoparticles were well dispersed and uniform in size**.**

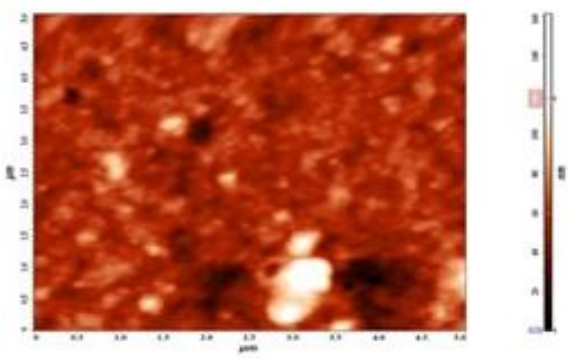
***Penicillium pinophilum* (MTCC 2192)**

This absorption band is called surface plasmon resonance which might have been involved in the alteration of the solution color due the excitation of surface plasmon vibrations of the nanoparticles [25, 26, 27]. The exact mechanism for the synthesis of silver nanoparticles were not known yet but several reports had confirmed that the extracellular reductase enzyme produced by fungi during the contact between its cell wall with silver resulting in the reduction of silver ions into silver nanoparticles [28, 29]. FTIR analysis was used for the identification of the molecules, proteins and functional groups involved in the reduction of silver ions into silver nanoparticles and was stabilized as a capping agent (Fig 3). The FTIR analysis obtained for the nanoparticles showed that the absorption peaks located at 3417.6 cm^{-1}(O-H stretch), 2923.8 cm^{-1}(C-H amide),1643.2 cm^{-1}(C═O amides),1542.9 cm^{-1}(N-H amides) 1380.9cm^{-1} (CH_3 bend of alkenes), 1234.3 cm^{-1} (C-O stretch),1072.3cm^{-1}(C-N Stretch of amines), 617.1 cm^{-1} **(**acetylenes C-H bend of alkynes).

***P. pinophilum* (MTCC-2192)**

The particles were further characterized by AFM to determine the size, agglomeration, surface roughness together. Two dimensional image of AFM showed the agglomeration and particle size while as three dimensional images showed the particle height and also

informed about the average roughness of the silver nanoparticles. It was observed that silver nanoparticles were agglomerated and polydispersed (Fig 4 and Fig 5). The particle size was found within the ranges from 40nm to 70nm.



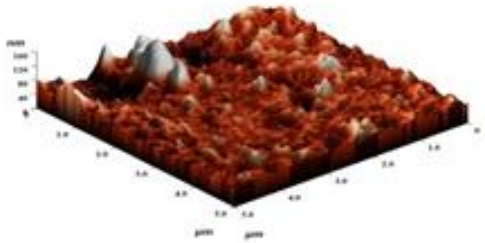


Fig 4 &. 5: 2D and 3D picture of Atomic Force microscopy (AFM) shows the particle height, roughness and inhomogenity of cluster formation of silver nanoparticles synthesized from *P. pinophilum* (MTCC 2192)

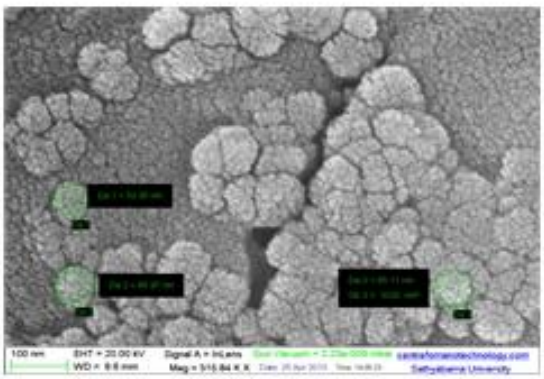


Fig. 6: Field emission Scanning electron microscope shows the particles are spherical and size of silver nanoparticles (33.29 to 66.97nm). Scale bar = 100nm

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

S. No.	Pathogenic Bacteria	Carbicillin (A)	Ag NPs	Carbicillin + AgNPs (B)	Increase in fold area (%)	Moxifloxacin (A)	Moxifloxacin + AgNPs (B)	Increase in fold area (%)
1	*S. aureus*	27	14	35	0.68	24	28	0.36
2	*Bacillus cereus*	29	14	34	0.37	21	29	0.90
3	*E. coli*	20	12	32	1.56	25	27	0.16
4	*Vibrio cholera*	26	13	37	1.0	28	37	0.74
5	*Proteus vulgaris*	24	11	29	0.46	25	28	0.25

***P. pinophilum* (MTCC 2192).**

Field emission scanning electron microscope were used to understand the size and morphology of the silver nanoparticles which showed the silver nanoparticles were well dispersed and spherical in their shapes in the range between 33.29 to 66.97nm

The antimicrobial activity of silver nanoparticles were analyzed through disc diffusion method against different pathogens viz., *Staphylococcus aureus, Bacillus cereus, E. coli, Vibrio cholera* and *Proteus vulgaris* and found satisfactory in the present study. The synergistic effect of silver nanoparticles with Carbicillin (100mcg disc) and Moxifloxacin (5mcg disc) were also analyzed. The highest increase in fold area was found the maximum for Carbicillin in presence of AgNPs against *E. coli* (1.56), followed by *Vibrio cholera* (1.0), *S. aureus* (0.68) and *Proteus vulgaris* (0.46). Likewise for Moxifloxacin, the increase fold area was observed highest against *Bacillus cereus* (0.90) followed by *Vibrio cholera* (0.74), *S. aureus* (0.36) and *Proteus vulgaris* (0.25) (Table 1). The results showed that the antibacterial activity of Carbicillin and Moxifloxacin in presence of nanoparticles was increased in combined formulation of silver nanoparticles. From the above investigation it was clear that silver nanoparticles synthesized from *Penicillium pinophilum* (MTCC 2192) possesses antibacterial potency noble drugs against these clinically isolated pathogens. The synergistic activity of silver nanoparticles enhanced the antibacterial property of Carbicillin and Moxifloxacin studied during the study period.

Increase in fold area was calculated by using the equation $(B^2-A^2)/A^2$, where A is the zone of inhibition of AgNPs and B is the zone of inhibition of antibiotic +AgNPs respectively.

CONCLUSION

During the present study, *Penicillium pinophilum* (MTCC 2192) was employed for the first time in order to synthesize the silver

nanoparticles by extracellular method. Occurrence of brown color of the solution indicated the formation of silver nanoparticles. Biological way of nanoparticle synthesis was found quite safe, ecofriendly and cost effective. The synthesized silver nanoparticles enhanced the antibacterial property of Carbicillin and Moxifloxacin against clinically isolated pathogens in combined formulation. Hence it may be assessed that combined formulation of available drugs with silver nanoparticles would be an alternative approach in order to treat the multi drug resistant pathogenic bacteria and also to minimize the antibiotic doses to cure the dreaded diseases. Moreover it may take time and needs animal trial to check its toxicity on human beings before bring about to the market as general drugs.

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