Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2015, 7 (5):281-285
(http://scholarsresearchlibrary.com/archive.html)

Der Pharmacia Lettre
Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Enhanced antibacterial activity of commercial antibiotics using AgNPs synthesized from *Aspergillus niger*

Anima Nanda[1], M. Amin Bhat[1] and *B. K. Nayak[2]

[1]Faculty of Bio & Chemical Engineering, Sathyabama University, Jeppiaar Nagar, Chennai, India
[2]Department of Botany, K.M. Centre for P.G. Studies (Autonomous), Pondicherry

---

ABSTRACT

Nanobiotechnology has emerged as an important branch of nanotechnology with biological or biochemical applications and activities at nano level in order to design or to study existing properties of nature. The biosynthetic utilization, especially fungi, has emerged as a novel method for the synthesis of metal nanoparticles. The metal nanoparticles are considered as fundamental molecular building blocks for nanotechnology. In the present study, Aspergillus niger was utilised for the extracellular synthesis of biogenic silver nanoparticles (AgNPs). The fungus mycelium is exposed to the 1mM silver nitrate solution that prompts the fungus to produce enzymes and metabolites for its own survival and reduces Ag^{+} ions into Ag^{0} due to catalytic effect of the fungal enzymes and metabolites. The primary confirmation of AgNPs synthesis was confirmed by the colour change from whitish colour to yellowish brown colour, which was confirmed through absorption UV-Visible spectrophotometer. The Fourier transform infrared spectroscopy analysis confirmed the quantitative analyses different reaction products revealed capping of silver nanoparticles. Field emission scanning electron microscopy (FESEM), Atomic force microscopy (AFM) analysis indicates that extracellular AgNPs are having average roughness and are in average size of 25-65nm. FESEM analysis showed AgNPs are spherical shaped and monodispersed. The biological Silver nanoparticles are known to have bactericidal effects against MDR strains of S. auras, B. cereus, E. coli, V. cholera and P. vulgaris. Resistance of bacterial infections has emerged in recent years has became major health problem. The bactericidal activity of biologically synthesised AgNPs was carried against pathogenic MDR strains in combination with commercial antibiotics which showed enhanced efficacy antibiotics against selected pathogens. Thus, the present study demonstrates that AgNPs in combination with antibiotics are showing potential bactericidal property could be used as powerful weapons against the MDR pathogens.

Key words: *Aspergillus niger*, UV-Vis Spectrophotometer, FTIR, AFM, FESEM, MDR

INTRODUCTION

Nanotechnology is emerging as a rapidly growing field with its applications in various fields such as industrial, biomedical and electronic etc. Nanoparticles of noble inorganic metals are being used and studied for its various applications in the field of nanoscience and nanotechnology. Noble metals such as Ag, Au, Pd, Pt and Cu have been widely used for the synthesis of nanomaterials which are useful in the various areas like optoelectronics[1], catalysis[2], Photo thermal therapy[3], surface enhanced Raman scattering (SERS) detection[4] and biological labelling[5]. The Nanoparticle research is inevitable today not because of only application but also by the way of synthesis[6], because nanoparticles are being synthesized by different methods like chemical, physical and newly developed biological methods having different applications. Among several inorganic metal nanoparticles, Silver nanoparticles (Ag-NPs) have been considered an important area of research due to their unique and intense plasmon resonance properties in the visible range and being used most widely from the ancient times to fight against various diseases. The Silver has long known history of having strong inhibitory and bactericidal effects as well as a broad spectrum of antimicrobial activities, even at low concentrations[7].

There is a growing need for the development of reliable experimental protocols for the synthesis of these metal nanomaterials over a range of chemical compositions, sizes, and high monodispersity which is one of the challenging issues in current nanotechnology. In this context of the current research drive is to develop green technologies in nanoparticle synthesis of considerable importance. Most of the techniques are costly as well as inefficient in the production of reliable materials and energy. Hence, there is a growing need to develop clean, nontoxic, and environmentally benign synthesis procedures. The silver nanoparticles can be synthesised by physical, chemical and biological methods but biological method are showing the importance because they cost effective, eco-friendly and has shown other advanced properties over physical and chemical methods[8, 9].In the green synthetic methods, various living organisms have been exploited for the biosynthesis silver nanoparticles like bacteria[10, 11], fungi [12-14], Actinomycetes [15] and plants [16]. The microbial biosynthesis of silver nanoparticles has shown potential impact in the field of nanotechnology because they are easy to synthesise, eco-friendly. The number of pathogenic and non pathogenic fungi has been exploited for the biosynthesis silver nanoparticles, which suggested that metal oxides (gold or silver) are being reduced to produce the stable nanoparticles[17]. The aim of the present study was to synthesize silver nanoparticles from *Aspergillus niger*. The synthesised AgNPs were characterised and evaluated for the antibacterial activity against the multi drug resistant clinical pathogens.

MATERIALS AND METHODS

Isolation of fungi

In the present study, *Aspergillus niger* was isolated from the aerial environment of Sathyabama University by exposing Sabouraud's Dextrose agar medium on Petriplates based on gravitation method. The exposed petriplates were brought and incubated for at 25°c for 3-7 days in the Microbiology Research Laboratory, Department of Biomedical Engineering, Sathyabama University. The fungal colony was identified based on the author's expertise and also by laboratory manuals. The pure culture of *Aspergillus niger* was kept in 4^{o}c in the refrigerator for the biosynthesis of silver nanoparticles.

Biosynthesis and characterization of AgNPs

The mould fungi *Aspergillus niger* was used for the biosynthesis silver nanoparticles. The nanoparticles were characterised through UV- Visible spectroscopy followed by FTIR, FESEM and AFM analyses following as per the same procedures of early reports [18].

Determination of bactericidal activity

The antibacterial activity of Bio-NPs was determined by performing antimicrobial susceptibility test by disc diffusion method against clinical pathogens such as *S. aureus, B. cereus, E. coli, P. vulgaris and Pseudomonas* sp. [19]. The clinical pathogens were grown on aerobically on nutrient agar medium (Hi-Media, Mumbai, India). The Sterile disc (6mm) and standard antibiotics (Amoxicillin and Carbenicillin) were purchased from Hi Media (Mumbai, India). The agar plates were prepared as directed by the manufacturer. The inoculums were prepared from fresh culture of the microbial strain, kept for 18-24hrs at 37^{o}C. 3-5 fresh colonies of microbial strain was inoculated into a tube containing 4- 5ml of freshly prepared Nutrient broth (Hi-Media) and incubated for 2-3hrs to standardize the culture to 0.5McFarland standards CFU/ml. The inoculums suspension with the help of swab was inoculated on Petri plates by streaking over the entire sterile agar plate's surface. The sterile discs were kept on solid agar in the centre along with the antibiotics, Amoxicillin and Carbenicillin. The efficacy of Bio-NPs were evaluated singly and in combination with freshly prepared Bio-NPs (1mg/ml Stock solution) were added to sterile discs, while as standard discs (Amoxicillin and Carbenicillin) on agar plates inoculated with pathogens were impregnated with 20µg solution of Bio-NPs and incubated at 37^{o}c for 18-24hrs[18]. The Amoxicillin and Carbenicillin discs were used as positive control and along with fungal Cell free filtrate as negative control. The zone of inhibition was measured and compared with the control. The experiments were repeated in tree times.

Statistical analysis

The experimental study was carried in triplicate and the representative data is presented in the research article. Arithmetic mean values were taken for antibacterial assays for considering the data analysis. T-test was performed for comparative analysis of unpaired data.

RESULTS AND DISCUSSION

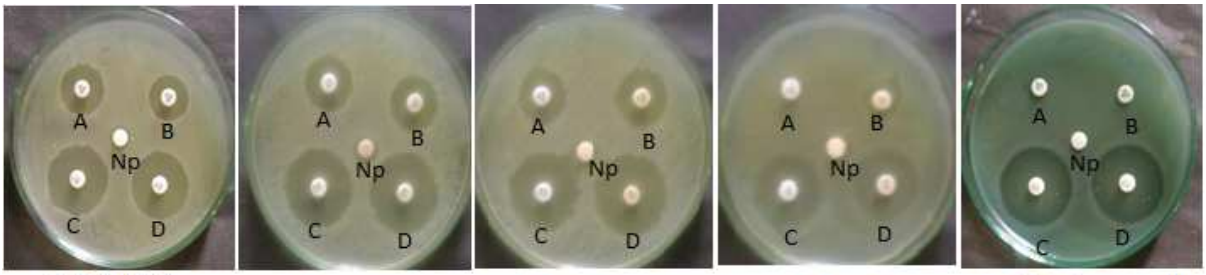
The mould fungi *Aspergillus niger* was used for the synthesis of silver nanoparticles. The nanoparticle synthesis was primarily confirmed by observing from the colour change, indicating the formation of silver nanoparticles. The AgNPs formation was confirmed UV-visible spectrophotometer analysis in the range of 350-700nm and showed absorption band 416nm, which is the characteristic range of silver nanoparticle synthesis. FTIR analysis confirms and identifies the biomolecules involved in the reduction of Ag^{+} ions into $Ag^{0.}$ While interpreting the FTIR peaks it

was concluded that Ag^+ ions were possibly responsible for capping and efficient stabilization of the metal nanoparticles synthesized by *Aspergillus niger* [20]. The average roughness and topography and morphology of biosynthesised silver nanoparticles were analysed by AFM and FESEM which were in the average range of 45-65nm. The Bio-AgNPs were distributed uniformly and having smooth surfaces and rough surfaces were spherical to ovate in structure with having average dimensional dimensions in size 50-65nm [18].

Bactericidal Activity

The antibacterial activity of AgNPs and their comparative analysis was determined by disc diffusion method. The freshly prepared AgNPs synthesised from *Aspergillus niger* were checked for their antibacterial activity against clinical pathogens viz., *Staphylococcus aureus, Bacillus cereus, E. coli,* and *Proteus vulgaris* were satisfactory in the present study. The AgNPs were evaluated for combined effects with commercial antibiotics like Amoxicillin (30mcg/disc) and Carbenicillin (100mcg/disc) showed satisfactory results against test pathogen (Fig1 and Table 1). While studying their comparative analysis the standard antibiotic disc (Amoxicillin and Carbenicillin) were impregnated with 20ul of AgNPs, while as cell free filtrate (Without $AgNo_3$) was taken as negative control. The standard antibiotic (Amoxicillin 30mcg/disc and Carbenicillin 100mcg/disc) were taken as positive control. The AgNPs have shown good activity against clinical pathogens, While as AgNPs in combined form along with Amoxicillin and Carbenicillin, showed significant increase in the zone of inhibitions. The zones of inhibitions were measured in mm along with their enhanced effect to confirm the combined effect of AgNPs along with commercial antibiotics against selected pathogens which is represented in (Table 1).

The freshly prepared AgNPs showed maximum antibacterial activity against *P. Vulgaris* followed by *S. aureus, B. Cereus, Escherichia coli and pseudomonas sp*. The commercial antibiotics Amoxicillin (30mcg/disc) and Carbenicillin 100mcg/disc were impregnated with a freshly prepared AgNPs 20µl/disk have shown remarkable enhancement in the zone of inhibition against clinical strains. The diameters of inhibition zones (mm) around the Amoxicillin with and without AgNPs shows enhanced antibacterial activity of against *S. aureus (26±0.33) followed by P. vulgaris* (25±0.63) *B. Cereus* (23±0.44), while as the *E. coli* and *Pseudomonas* sp. have not shown any inhibition against Amoxicillin but after adding Bio-NPs to Amoxicillin *E. Coli* (12±0.84) showed good zone of inhibition while as *Pseudomonas sp* does not shows any effect. While as Carbenicillin with and without Bio-AgNPs are equally effective against clinical pathogens and is showing enhanced efficacy due to Bio-NPs (Fig 1).



a. S. aureus *b. B. cereus* *c. P. vulgaris* *d. E. coli* *e. Pseudomonas* Sp.

Fig 1: Antibacterial effect of Bio-AgNPs along with Amoxicillin and Carbenicillin against test pathogens

Thus AgNPs synthesised from *Aspergillus niger* showed good antibacterial activity against clinical pathogens and also enhances antimicrobial activity of Amoxicillin and Carbenicillin. These type of reports have been also shown by some earlier workers in which biologically synthesised silver nanoparticles not only shows good antibacterial activity but also enhances the efficacy of antibiotics [13, 18, 21, 22]. This type of study was carried first time in which mould fungi *Aspergillus niger* has been exploited for the biosynthesis of AgNPs to see its combined effect with Amoxicillin and Carbenicillin hence these type of results could be used to control the drug dosage which is becoming the major world threat due to multi drug resistance to antibiotics but needs further studies.

Table 1: Comparison of antibacterial activity of silver nanoparticles alone and in combination with antibiotics, Amoxicillin (30mcg/disc) and Carbenicillin (100mcg/disc) against pathogenic bacteria

Pathogens	Bio-NPs	AMX	AMX + Bio-NPs	CARB	CARB+ Bio-NPs
	Zone of Inhibition(mm)				
S. aureus	11±0.23	21±0.38	26±0.33	*23±0.38*	31±0.16
B. cereus	10±0.32	17±0.42	23±0.44	*22±0.34*	29±0.48
P. vulgaris	12±0.26	18±0.32	25±0.63	22±1.36	31±0.27
E. coli	10±0.44	.....	12±0.84	*21±1.82*	29±0.68
***Pseudomonas* Sp.**	09±0.51	...	.....	24±0.28	31±0.21

**Bio-NPs= Silver Nanoparticles, AMX. = Amoxicillin (30mcg/disc), CARB= Carbenicillin (100mcg/disc)*

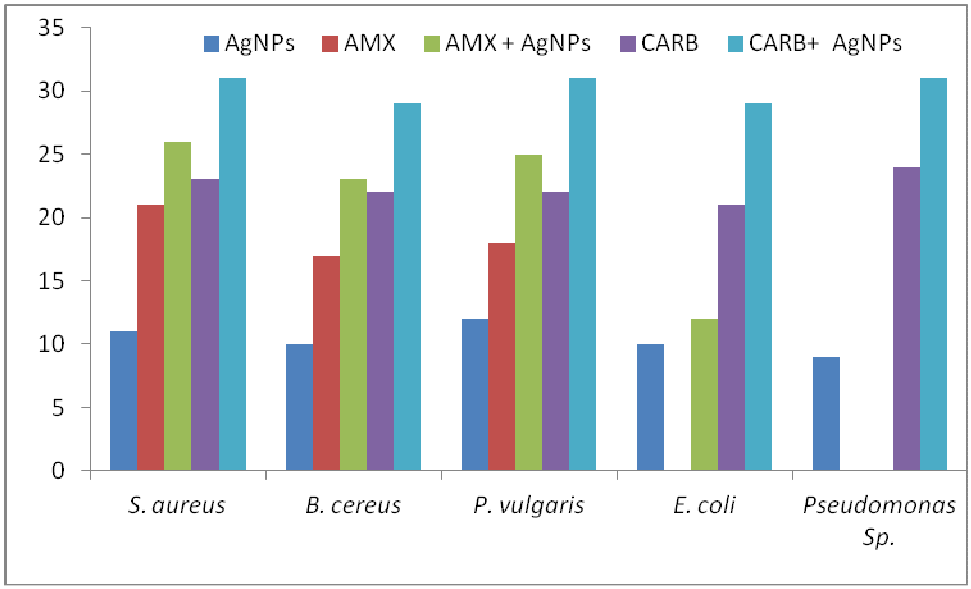


Fig 2: Graphical representation of combined effect Amoxicillin and Carbenicillin with AgNPs against test pathogens

CONCLUSION

During the present study, *Aspergillus niger* was used for the synthesis of silver nanoparticles which was found simple and environment friendly. The bionanoparticles synthesis was confirmed through UV-visible spectrophotometer and characterized by the instruments like, FTIR, AFM and FESEM. The nanoparticle synthesized was spherical to ovate in shape and were polydispersed in nature. The AgNPs showed good antibacterial activity against clinical pathogens and while in combination with commercial antibiotics (Amoxicillin and Carbenicillin) the antibacterial activity was further enhanced. Thus, we can conclude that the silver nanoparticles can be used as an antibacterial material alone and also in combination with the antibiotics but needs further studies to find out the mechanism behind the action of AgNPs on standard antibiotics and its enhanced bactericidal activity.

Acknowledgments
The authors would like to acknowledge DBT, Government of India, New Delhi for the financial assistance, Department of Biomedical Engineering and to the Sathyabama University, Chennai, India for providing the necessary facilities to carry out this research.

REFERENCES

[1] M. Tamura and H. Fujihar, *J. Am. Chem. Soc.* **2003**, 125 (51), 15742-15743.
[2] L. Jia, Q. Zhang, Q. Li and H. Song, *Nanotechnology*. **2009,** 20 (38), 385601.
[3] P. K. Jain, X. Huang, I. H. El-Sayed and M.A. El-Sayed, *Acc Chem Res.* **2008**, 41(12), 1578-1586.
[4] Y. Liu, J. Hu, Q. Kong and X. Feng, *Mater Lett*, **2010**, 64, 422–424.
[5] C. Leng, J. Wu, Q. Xu, G. Lai, H. Ju and F. Yan F, Biosens Bioelectron, **2011**, 27(1), 71-76.
[6] V. Gopinath, D. MubarakAli, S. Priyadarshini, N. M. Priyadharsshini, N. Thajuddin and P. Velusamy, *Colloids and Surfaces, B: Biointerfaces*, **2012**, 96, 69-74.
[7] J. R. Morones, J. L. Elechiguerra, A. Camacho et al., *Nanotechnology*, **2005**, 16, 2346–2353.
[8] S. G. David, Bionanotechnology: lessons from nature. New York: Wiley, **2004**.
[9] S. Talebi, F. Ramezani, M. Ramezani, *Nanocon Olomouc, Czech Republic, EU*, **2010**, 10, 12-18.
[10] A. Nanda and M. Saravanan, *Journal of Nanomedicine: Nanotechnology, Biology and Medicine*, **2009**, 5, 452–456.
[11] S. Pal, Y.K. Tak, Song J M, *Appl Environ Microbiol*, **2007**, 73, 1712-1720.
[12] M. Saravanan and A. Nanda, *Colloids and Surfaces B: Biointerfaces*, **2010**, 77, 214-218.
[13] M. A. Bhat, B. K. Nayak, A. Nanda, *Journal of Pure and Applied Microbiology*, **2014,** 8 (5), 4201-4207.
[14] B. K. Nayak, M. A. Bhat, A. Nanda, Journal of Chemical and Pharmaceutical Sciences, 2014, 2, 86-89.
[15] A. Zarina, A. Nanda, *J. Pharm. Sci. & Res.* **2014**, 6(10), 321-327.
[16] J. Kesharwani, J. Hwang, K. Yoon and M. Rai, *J Bionanosci.***2009,** 3, 39-44.
[17] A. Ingle, Gade A, Pierrat S, Sönnichsen C and Rai M, *Curr Nanosci.* **2008**, 4, 141-144.
[18] M.A. Bhat B. K. Nayak and A. Nanda, *Int J Pharm Bio Sci*, **2015**, 6(1), 506 – 515.
[19] A.W. Bauer, M. Kirby, Sherris J.C, and M. Truck, *Am J Clin Pathol*, **1966**, 51, 493-496.

[20] K.S. Naveen, G. Kumar, L. Karthik, K.V Rao, *Archives of Applied Science Research*. **2010**, 6, 161-167.
[21] A. D. Mudasir, A. Ingle and M. Rai, *Nanomedicine: Nanotechnology, Biology and Medicine*, **2013**, 9, 105–110.
[22] S.S. Birla, V.V. Tiwari, A.K. Gade, A.P. Ingle, A.P. Yadav and M.K. Rai, *Lett Appl Microbiol,*. **2009**, 48, 173-179.