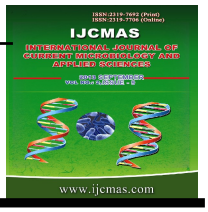
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 2 Number 9 (2013) pp. 223-230**
http://www.ijcmas.com



Original Research Article

Antimicrobial activity of extracellularly synthesized silver nanoparticles from marine derived actinomycetes

S.Deepa*, K. Kanimozhi and A.Panneerselvam

PG and Research Dept. of Botany and Microbiology, A.V.V.M Sri Pushpam College, (Autonomous), Poondi – 613 503, Thanjavur, Tamilnadu, India
**Corresponding author e-mail: sdeepa30@gmail.com*

Keywords

Antibacterial activity; Marine sediment; silver nanoparticles; FT-IR; SEM.

A B S T R A C T

The process of development of reliable and eco-friendly metallic nanoparticles is an important step in the field of nanotechnology. To achieve this use of natural sources like biological systems becomes essential. In the present work, we have investigated extracellular biosynthesis of silver nanoparticles of AgNO3 using *Thermoactinomyces* sp. isolated from the marine sediment samples of Vellappallam Coast, Nagapattinam Dt, Tamil Nadu, India. Biosynthesized silver nanoparticles were confirmed by UV – visible spectroscopy, the spectra showed a maximum absorption peak at 410 nm and the FT-IR analysis, provides evidence SEM confirmed the silver nanoparticles were spherical in shape and size in the range of 20-40 nm. The antimicrobial activities of silver nanoparticles were screened against common human pathogen (*Bacillus subtilis, Enterobacter aerogenes, Enterococcus faecalis, Staphylococcus aureus* and *Streptococcus pyogenes*) and five gram negative bacteria (*Escherichia coli, Klebsiella oxytoca, K. pneumoniae, Salmonella typhi,* and *Vibrio cholerae*). The results showed silver nanoparticles synthesized of AgNO3 displayed a significant antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis.*This study gives an innovative approach to develop new formulations based on metallic nanoparticles with antimicrobial properties to reach the pharmaceutical companies searching for new unconventional antibacterial agents.

Introduction

Nanotechnology is an emerging and rapidly growing field with its application in science and technology for the purpose of manufacturing new materials at the nano scale level (Albrecht *et al*., 2006). Nanotechnology is a field that is burgeoning day by day making an impact in all spheres of human life (Vaidyanathan *et al*., 2009). The word **"**Nano**"** is used to indicate one billionth of a meter or 10-9. The term nanotechnology was coined by Norio Taniguchi, a researcher at the University of Tokyo, Japan (Taniguchi, 1974).

"Nanotechnology**"** is the application of science to control matter at the molecular level. It is the most promising field for

generating new applications in medicine. Silver nanoparticles have an important advantage over conventional antibiotics in that they kill all pathogenic microorganisms, no organisms has ever been reported to readily develop resistance to it. In the last few decades there has been increased interest in reducing the availability of commercial textile containing antibacterial agents due to environmental pollution.

Since silver is a good antibacterial agent and non-toxic and natural inorganic metal, it appears as an interesting material to be used in different kind of textile fibers. In this direction, polypropylene/silver nano composite fibers were prepared and the antibacterial tests showed that the fibers containing silver nanoparticles in core-part (inside the fiber) had no nearly significant antibacterial activity. However, the fibers having silver nanoparticles (30 nm size) in sheath-part showed excellent antibacterial effects. (Lee, 2003).

Silver nanoparticles have unique optical properties because they support surface plasmon resonance and also display unique chemical properties. Actinobacteria are Gram positive, aerobic and high G+C content bacteria. They are one of the major groups of soil population and are widely distributed (Kuster, 1968).

New technology advances in reducing silver compound chemically to nanoscale sized particles have enabled the integration of this valuable antimicrobial into a larger number of materials - including plastics, coatings, and foams as well as natural and synthetic fibers. Nano-sized silver have already provides a more durable antimicrobial protection, often for the life of the product.

Current research in inorganic nanomaterials having good antimicrobial properties has opened a new era in pharmaceutical and medical industries. Silver is the metal of choice as they hold the promise to kill microbes effectively. Silver nanoparticles have been recently known to be a promising antimicrobial agent that acts on a broad range of target sites both extracellularly as well as intracellarly.

Silver nanoparticles shows very strong bactericidal activity against gram positive as well as gram negative bacteria including multiresistant strains (Shrivastava *et al.,* 2007), and also it was found to be in few studies (Zeng *et al*.,2007; Roe *et al*., 2008). Hence there is a huge scientific progress in the study of biological application of ZnO and Ag and other metal NP.

Actinobacteria will provide a valuable resource for novel products of industrial interest, including antimicrobial agents (Mitsuiki *et al*., 2002). The aim of this study is to synthesize the silver nanoparticles using *Thermoactinomyces* sp. and to characterize by using various instruments such as, UV – Vis spectroscopy, FTIR, and SEM.

Materials and Methods

Sample collection

The present investigation was carried out by collection and examination of mangrove soil samples from four different seasonal variations were collected from mangrove environment of Vellappallam, Nagapattinam Dt, Tamil Nadu, India. The collected samples were carefully stored in polythene bags and transported to the laboratory for the further uses.

Isolation of actinomycetes

Isolation of actinobacteria was performed by serial dilution and plating technique using starch casein agar medium. One gram of this soil sample was suspended in 25 ml sterile water in a conical flask, stirred thoroughly with the help of a glass rod and left for some time. Distilled water (9ml) was taken in each of the 7 test tubes and labelled from 1 to 7. The supernatant liquid from the dissolved soil sample was transferred into the test tubes so as to achieve the serial dilutions of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}. 1 ml of the diluted sample was inoculated in the starch casein agar medium plates from each dilution. The Petriplates are then rotated to spread the sample uniformly. Plates were then incubated at room temperature (28 to 30ºC) for 7 days.

Screening of Actinomycetes for antibacterial efficacy

The selected actinomycetes were screened for antibacterial efficacy by agar well diffusion method. The five gram positive bacteria (*Bacillus subtilis, Enterobacter aerogenes, Enterococcus faecalis, Staphylococcus aureus* and *Streptococcus pyogenes*) and five gram negative bacteria (*Escherichia coli, Klebsiella oxytoca, K. pneumoniae, Salmonella typhi,* and *Vibrio cholerae*) were obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India.

Synthesis of silver nanoparticle from actinomycetes

The selected actinomycetes mats were washed thrice in deionized water to remove the unwanted material. Approximately 3.5 gm of actinomycetes mats taken in a conical flask containing 100ml deionized water. 10^{-1}mM $AgNO_3$ was added then it was incubated 37^{o}C for 3 days. After incubation period colour change was observed.

SEM analysis of silver nanoparticles synthesized by actinomycetes

Sample preparation

Silver nanoparticle synthesized actinomycetes mats were allowed to dry completely and ground well. Since the specimen was at high vacuum, Fixation was usually performed by incubation in a solution of a buffered chemical fixative gluteraldehyde. The dry specimen was mounted on a specimen stub using an adhesive epoxy resin or electrically-conductive double-sided adhesive tape and sputter coated with gold palladium alloy before examination in the microscope.

FT –IR analysis of silver nanoparticles synthesized by actinomycetes

A known weight of sample (1 mg) was taken in a mortar and pestle and ground with 2.5 mg of dry potassium bromide (KBr). The powder so obtained was filled in a 2 mm internal diameter micro-cup and loaded onto FTIR set at 26°C ± 1°C. The samples were scanned using infrared in the range of 4000–400 cm-^{1} using Fourier Transform Infrared Spectrometer (Thermo Nicolet Model-6700). The spectral data obtained were compared with the reference chart to identify the functional groups present in the sample.

UV – Visible spectroscopy analysis of silver nanoparticles synthesized by actinomycetes

The reduction of silver ions was monitored by measuring the UV-VIS spectrum of the

reaction medium at 24 hrs time interval by drawing 1cm of the samples and their absorbance was recorded at a resolution of 0.5m at 350-800nm using UV-VIS spectrophotometer – UV 450 (Shimadzu).

Results and Discussion

In general, totally 56 actinomycetes species belonging to 25 genera were isolated from the mangrove soil samples Vellappallam mangrove forest, Nagapattinam District. Besides the above, maximum number of species diversity was encounted with the actinomycetes species belonging to the class *Streptomyces* (15), *Actinopolyspora* (10), *Actinomadura*(5), *Nocardiopsis*(10), *Micromonospora*(10) and *Actinomyces*(6).

Among actinomycetes, the members of the genus *Streptomyces* are considered economically important because they alone constituted 50% of the total soil actinomycetes population (Xu *et al.,* 1996) and 75% of total bioactive molecules are produced by this genus (Demain, 2000). The streptomycetes produce an array of secondary metabolites such as enzyme inhibitors, herbicides and large number of antibiotics (Omura, 1992; Lange and Sanchez Lopez, 1996; Demain, 1999).

Antibacterial efficacy of *Thermoactinomycetes* sp.

Diethyl ether extract exhibited minimum to moderate activity against the tested pathogens (Inhibition zone ranging 6.5 to 11.3 mm). Distilled water extracts showed least activity against *Enterococcus faecalis* and *Escherichia coli.* The ethyl acetate extract of *Thermoactinomycetes* sp. exhibited maximum inhibition zone of 16.3 mm and 16.1 mm against *Staphylococcus aureus* and *Bacillus subtilis* respectively. Ethyl acetate extract showed promising antibacterial activity when compared to other extracts (Table: 1).

The isolation of the antibacterial metabolite mildiomycin from a culture of *Streptoverticillium rimofaciens* (Iwasa *et al*., 1978). Mildiomycin is strongly active against several powdery mildews on various crops (Harada and Kishi 1978), acting as an inhibitor of the fungal protein biosynthesis (Feduchi *et al.,* 1985).

Biosynthesis of silver nanoparticles by *Thermoactinomyces* sp.

In this study, silver nanoparticles were synthesized using a reduction of aqueous Ag^{+} with the mat extracts of *Thermoactinomyces* sp. at room temperature. It was generally recognized that silver nanoparticles produced brown solution in water, due to the surface plasmon resonances (SPR) effect and reduction of $AgNO_3$. After the addition of $AgNO_3$ solution, the actinomycetes mat extracts of selected actinomycetes changed from light pink to dark pink colour in a few hours, while no colour change was observed in the mat extract without $AgNO_3$. Thus, colour change of the solution clearly indicated the formation of silver nanoparticles.

The production of pyramidal and 5-200 nm sized silver nanoparticles by *Phaenerochaete chrysosporium* was reported (Vigneshwaran *et al.,* 2006), whereas *Coriolus versicolor* (Sanghi and Verma, 2009) produced spherical and 25-75 nm sized particles, and *Penicillium brevicompactum* synthesized spherical shaped particles of 58.35±18nm size which indicated that the biochemical and genetic nature of microbial strain

Table.1 Antibacterial activity of *Thermoactinomyces* sp

S. No	Bacterial Pathogens	Zone of inhibition (diameter in mm) (Concentration - 100mg/ml)		
		Diethyl ether	Distilled water	Ethyl acetate
1.	*Bacillus subtilis*	11.3±1.5	-	16.1±1.4
2.	*Enterobacter aerogenes*	10.3±0.7	-	14.6±1.8
3.	*Enterococcus faecalis*	9±0.9	5.6 ± 0.5	15±1.5
4.	*Escherichia coli*	7±1.3	2.9 ± 1.0	12.7±1.0
5.	*Klebsiella oxytoca*	9±1.6	-	11.9±1.3
6.	*K. pneumoniae*	7.6±2.0	-	13.3±1.2
7.	*Salmonella typhi*	6.5±1.8	-	10.7±1.1
8.	*Staphylococcus aureus*	10.3±1.0	-	16.3±1.5
9.	*Streptococcus pyogenes*	7±0.59	-	10.5±1.0
10.	*Vibrio cholerae*	-	-	11.6±2.0

Figure.1 Ultraviolet-Visible (UV-Vis) Spectroscopy

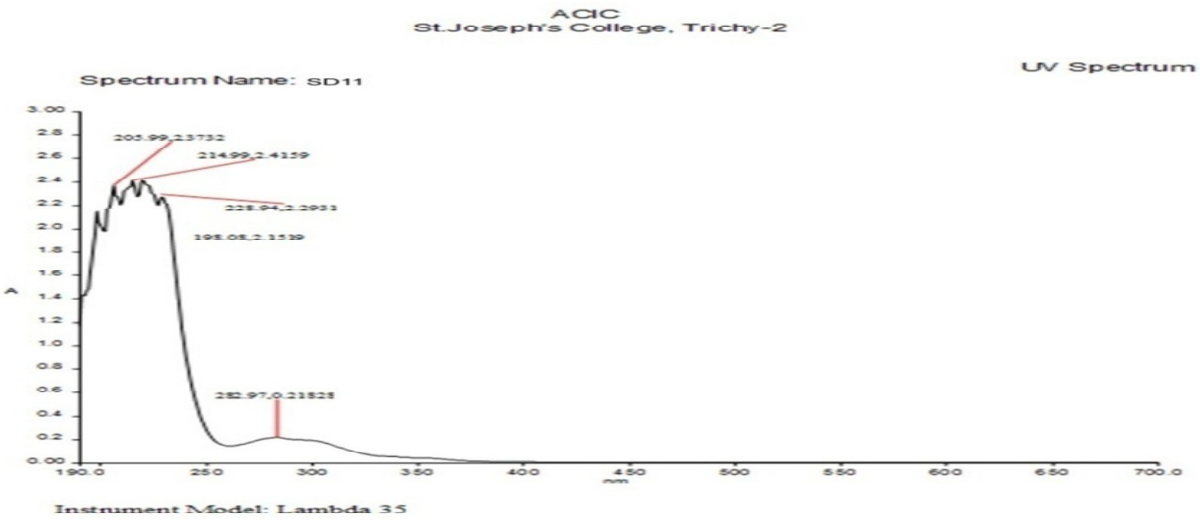


Table.2 FT – IR of silver nanoparticle synthesized by *Thermoactinomyces* sp

S.No	Group frequency cm^{-1} of the sample	Functional group assignment
1.	3411.33	Primary, bonded two bands
2.	1369.14	Alkane, $-CH_3$
3.	1233.23	Sulfites
4.	697.26	Alkene

Figure.2 FT – IR spectrum of silver nanoparticle synthesized by *Thermoactinomyces* sp.

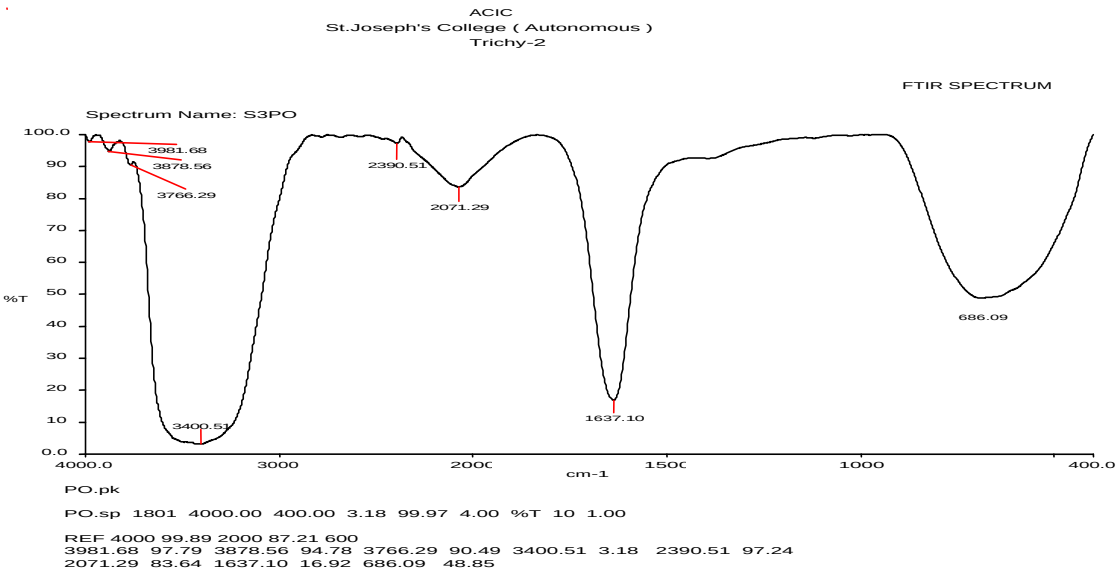
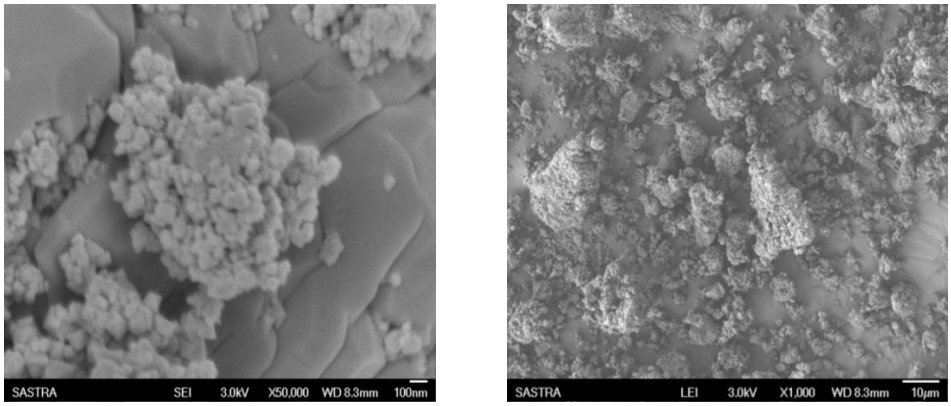


Figure.3 SEM analysis of silver nanoparticle synthesized by *Thermoactinomyces* sp.



employed plays a significant role in controlling the nanoparticle biogenic processes (Hemanth Naveen *et al.*, 2010).

Characterization of silver nanoparticles by *Thermoactinomyces* sp.

Ultraviolet - Visible (UV-Vis) Spectroscopy

All these reactions were monitored by ultraviolet-visible spectroscopy of the colloidal silver nanoparticles solutions. The ultraviolet-visible spectra of the *Thermoactinomyces* sp. mats with silver nanoparticles showed strong peaks at 320 - 410 nm range, which indicated the presence of silver nanoparticles. Curve 1 corresponds to incubation with silver nitrate after 48 hrs, 72 hrs and 96 hrs respectively. After 96 hrs of incubation, no change in intensity was observed indicating complete reduction of silver ions (Fig:1& 2).

Scanning Electron Microscopy (SEM) analysis of silver nanoparticle synthesized by *Thermoactinomyces* sp.

The SEM micrographs of the present study were taken at different magnifications. The silver nanoparticles synthesized by *Thermoactinomyces* sp. depicts that the SEM images of the silver nanoparticles at 2000X, 5000X and 10,000X magnifications. The nanoparticles were in the size ranging from 20-30 nm. The morphology of the nanoparticles was highly variable (Fig: 3)

Silver nanoparticles with mean diameters of 9, 11, 24 and 30 nm were synthesized using $AgNO_3$. The nanoparticles were characterized by UV/Vis, FT-IR and SEM. UV/Vis spectra show the characteristic plasmon absorption peak for the silver nanoparticles ranging from 320 to 410 nm. Additionally, the antibacterial activity of the nanoparticles dispersion was measured by agar well diffusion method. The advantage of using actinomycetes for the synthesis of nanoparticles is that they are easily available, safe to handle and possess a broad variability of metabolites that may aid in reduction. Moreover, these particles have innumerable applications.

Acknowledgement

We gratefully acknowledge the research and technical support provided by the college and the department throughout the work.

References

Albrecht, M.A., C.W. Evan, Raston, and C.R. 2006. Green chemistry and the health implications of nanoparticles. Green chem. 8: 417-432.

Vaidyanathan, R., K. Kalimuthu, G. Gopalram and Gurunathan, S. 2009. Nanosilver- The burgeoning therapeutic molecule and its green synthesis. Biotechnol. Adv. 27: 924-937.

Taniguchi, N., 1974. Proc. Intl. Conf-Prod. (ICPE). Tokyo, Japan.pp. 18-23.

Kuster, E., 1968. The Actinomycetes. *In*: *Soil Biology*,eds. Burgers (A) and Raw (F), Academic press, London.pp. 111-124.

Mitsuiki, S., M. Sakai, Y. Moriyama, M. Goto and Furukawa, K. 2002. Purification and some properties of keratinolytic enzyme from an alkaliphilic *Nocardiopsis sp*. Biosci. Biotechnol. Biochem. 66: 164-167.

Porter, J.N., J.J. Wilhelm Tresner, H.D. 1960. A Method for the preferential isolation of actinomycetes from soil. Appl. Microbial. 8: 174-178.

Eo, S.Y.Y., H.J. Lee, and Jeong,S.H. 2003. Preparation of nano composite fibers for permanent antibacterial effect. J. Mater. Sci. 38: 2143.

Eo, S.Y.Y., and Jeong. S.H. 2003. Preparation and characterization of polypropylene/silver nanocomposite fibers. Polymer Intern. 52: 1053.

Shrivastava, S., T. Bera, A. Roy, G. Singh, P. Ramachandrarao and Dash, D. 2007. Characterization of enhanced antibacterial effects of novel silver nanoparticles. Nanotechnol. 18: 9-12.

Zhang, L., Y. Jiang, M. Povey and York, D. 2007. Investigation into the antimicrobial behavior of suspension of ZnO nanoparticles (ZnO nanofluids). J. Nanoparti. Res. 9: 479-489.

Roe, D., B. Karandikar, N. Bonn-Savage, B. Gibbins and Roullet, J.B. 2008. Antimicrobialsurface functionalization of plastic catheters by silver nanoparticles. J. Antimicro. Chemo. 61: 869–76.

Demain, A.L., 1999. Pharmaceutically

active secondary metabolites of microorganisms. Appl. Microbiol. Biotechnol. 52: 455–463.

Demain, A.L., 2000. Small bugs, big business: the economic power of the microbe. Biotechnol. Adv. 18: 499–514.

Feduchi, E., M. Cosin and Carrasco, L. 1985. Mildiomycin: a nucleoside antibiotic that inhibits protein synthesis. J. Antibiot. 38: 415-419.

Harada, S., and Kishi, T. 1978. Isolation and characterization of mildiomycin, a new nucleoside antibiotic. J. Antibiot. 31: 519.

Iwasa, T., K. Suetomi and Kusuka, T. 1978. Taxonomic study and fermentation of producing organism and antimicrobial activity of mildiomycin. J. Antibiot. 31: 511-518.

Hemanth Naveen, K., S.Gaurav Kumar, L. Karthik and Bhaskara Rao, K.V. 2010. Extracellular biosynthesis of silver nanoparticles using the filamentous fungus *Penicillium* sp. Arch. Appl. Sci. Res. 2: 161-167.

Omura, S., Y.Tanaka, Y. Takahashi, I. Chias and Inoue, M. 1984. Irumamycin, an antifungal 20 membered macrolide produced by *Streptomyces:* Taxonomy fermentation and biological properties. J. Antibiot. 37: 157–1578.

Sanghi, R., and Verma, P. 2009. Biomimetic synthesis and characterization of protein capped silver nanoparticles. Biores. Technol. 100: 501-504.

Vigneshwaran, N., N. Arati Kathe, P.V. Varadarajan, P. Rajan Nachane and Balasubramanya, R.H. 2006. Biomimetics of silver nanoparticles by white rot fungus. *Phaenerochaete chrysosporium*. Colloids. Surf. B Interface. 53: 55-59.