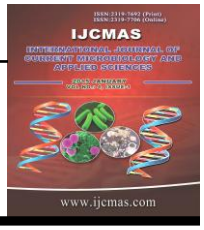
Int.J.Curr.Microbiol.App.Sci (2015) 4(3): 1073-1083

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 3 (2015) pp. 1073-1083**
http://www.ijcmas.com



Original Research Article

Biosynthesis of silver nanoparticles using soil Actinomycetes Streptomyces sp

K. Saminathan*

[1]Centre for Nanoscience and Technology, Tiruchengode, Tamilnadu, India

**Corresponding author*

Keywords

Silver nanoparticles, Streptomyces species, Antimicrobial activity, Synthesis

A B S T R A C T

The work is being carried out for the applications which would help in the prevention of human pathogens. We describe the synthesis of silver nanoparticles using Actinomycetes Streptomyces sp. A strong characteristic absorbance peak at around --- nm was observed at different time intervals. Particle size analysis of these particles shows that they are 70-100 nm in range and also this paper deals with a thorough investigation on the characterization of the silver nanoparticles by UV Visible, XRD, SEM and FTIR analysis and result reveals that, the average grain size of the silver nanoparticles formed in the bioreduction process is determined by XRD pattern of the silver nanoparticles formed in the experiment and is estimated to be greater than 50 nm and spherical in shape. FTIR spectral analysis showed array of absorbance bands in 400 cm^{-1} - 1500 cm^{-1} finally tested the biocompatibility by antimicrobial test against multi drug resistant human pathogens like *Escherichia coli, Staphylococcus aureus, Proteus vulgaris, Salmonella typhi, Klebsiella pneumonia, Shiegella dysentriae* and *Staphylococcus aureus.* The evaluation of agar diffusion test was made on the basis of zone of inhibition of bacteria around the test sample. The result reveals that the maximum antimicrobial activity was observed for *Escherichia coli.*

Introduction

The use of nanoparticles is important, as several pathogenic microorganisms have developed resistance against various antibiotics. Since noble metal nanoparticles such as gold, silver and platinum nanoparticles are widly applied to human contacting areas, there is a growing need to develop environmentally friendly processes of nanoparticles synthesis using microorganism and plant extracts (Kathireswari et al. 2014). Both unicellular and multicellular organisms are known to produce inorganic materials either intra or extracellularly (Ahmad et al., 2003; Mann, 1993), often of nanoscale dimensions and of exquisite morphology and hierarchical assembly. While the use of non-biological methods involves abnormal reaction conditions, high energy input and hazardous materials. Utilization of biological systems as nanofactories, promises an environmental benign, clean, nontoxic and economical

process. The biological and medical research communities have exploited the unique properties of nanomaterials for various applications (e.g., contrast agents for cell imaging and therapeutics for treating cancer). The size of nanomaterials is similar to that of most biological molecules and structures; therefore, nanomaterials can be useful for both in vivo and in vitro biomedical research and applications.

Silver is a well known antimicrobial metal capable to inhibit and kill bacteria and fungi .silver at nanoscale or nanoparcticles providing a greater surface area than microparcticles,then nanoparcticles provide higher availability of biocidal silver ions for improved antimicrobial activity. Silver based antimicrobial coatings are progressively used for different finishing or coating processes in medical device, textile and filtration, paper and packaging industries**.** Biologically synthesized silver nanoparticles have been known for their inhibitory and bactericidal effects and have various medical applications. While these nanoparticles are used to control or limit the growth of bacteria or fungi (inhibition), most of them do not provide sufficient silver ions to kill effectively and, therefore, to act as a biocide. In addition, for many applications, nanoparticles must be encapsulated in order to provide durability of antimicrobial properties. The encapsulation process must be carefully chosen and optimized in order to obtain durability of antimicrobial properties and provide sufficient silver ions to achieve significant kill rates. Recently, several antibacterial agents of textiles based on metal salt solutions ($CuSO_4$ or $ZnSO_4$) have been developed. Moreover, a new generation of dressing incorporating antimicrobial agents like silver and iodine have been studied (Schaller *et al.,* 2004). Antibacterial property of silver nanoparticles: currently silver coated dressings are extensively used for wound management particularly in burn wounds, chronic leg ulcers, diabetic wounds and traumatic injuries. Surface enhanced Raman Scattering using silver nanoparticles. Both unicellular and multicellular organisms are known to produce inorganic materials either intra or extracellularly (Ahmad et al., 2003a; Mann, 1996), often of nanoscale dimensions and of exquisite morphology and hierarchical assembly. Although nanomaterials are synthesized using physical and chemical approaches, it is now possible to synthesize nanomaterials using biological system with the help of microorganisms and plants. An important area of research in nanotechnology deals with synthesis of nanoparticles of different chemical compositions, sizes and controlled monodispersity. Currently, there is a growing need to develop environmentaily benign nanoparctical synthesis processes that donot use toxic chemicals in synthesis protocol.As a result ,researchers in the field of nanoparcticle synthesis and assembly have turned to biological systems for inspiration.Microorganisms play an important role in toxic metal remidiation through reduction of metal ions, this was considered interesting as nanofactories. Exposure of the Streptomyces biomass to aqueous Ag+ ions leads to extracellular reduction of metal ions and formation of silver nanoparticals. The Actinomycetes are present in marine & terrestrial environment and possessing properties intermediate between bacteria & fungi. They were originally designated as ‘ray fungi’. Focus on actinomycetes has primarily centerd on their phenomenal ability to produce secondary metabolites such as antibiotics. Streptomyces is the largest genus of Actinobacteria and the type genus of the family. Over 500 species of Streptomyces bacteria have been described.

Streptomycetes are gram-positive and have genomes with high GC-content. Streptomyces species are found worldwide in soil and are important in soil ecology. Streptomyces species produce spores from aerial filaments called sporophores. These rise above the colony and form spores called conidia by simple cross-wall divisions of the filament. Streptomycetes are metabolically diverse and can "eat" almost anything, including sugars, alcohols, amino acids, organic acids, and aromatic compounds. This is achieved by producing extracellular hydrolytic enzymes. There is considerable interest in these organisms as agents for bioremediation. Streptomycetes are characterised by a complex secondary metabolism , Streptomyces causes white grain mycetoma disease. Streptomycetes is the largest antibiotic producing genus, producing both antibacterials and antifungals, and also a wide range of other bioactive compounds such as immunosuppressants. Over 50 different antibiotics have been isolated from Streptomycetes species, including streptomycin, neomycin, chloramphenicol and tetracyclines. Using actinomycetes for synthesis of nanoparticles could be advantageous over other environmentally benign biological process of maintaining cell cultures. It can also be suitably scaled up for large scale synthesis of nanoparticles. It has been observed that the extremophilic actinomycete, *Thermomonospora* sp. when exposed to gold ions reduced the metal ions extracellularly, yielding gold nanoparticles with a much improved polydispersity (Sastry *et al*. 2003). More than 23 thousand bioactive microbial products including eight thousand anti infective were demonstrated the increasing relevance, so called actinomycetes are source of antibiotics (Lazzarini et al, 2000). Streptomycetes is the largest antibiotic producing genus, producing both antibacterials and antifungals, and also a wide range of other bioactive compounds such as immunosuppressants. Over 50 different antibiotics have been isolated from Streptomycetes species, including streptomycin**,** neomycin**,** chloramphenicol and tetracyclines.

Using actinomycetes for synthesis of nanoparticles could be advantageous over other environmentally benign biological process of maintaining cell cultures. It can also be suitably scaled up for large scale synthesis of nanoparticles. It has been observed that the extremophilic actinomycete, *Thermomonospora* sp. when exposed to gold ions reduced the metal ions extracellularly, yielding gold nanoparticles with a much improved polydispersity (Sastry *et al*. 2003).

Materials and Methods

The Streptomyces culture was collected from microbiology laboratory at the KSR college of Arts & Science, Thiruchengode. The collected sample was then brought to the laboratory for analysis within two hours. The actinomycetes was identified to according to the standard microbiological techniques.The sample was transferred to appropriate media like starch casein nitrate agar, starch casein agar, Bennett agar media. The Starch casein nitrate agar plates were prepared and collected cultures were streaked to obtain pure cultures, then incubated at 28^{o}C for 6-7 days. The grown cultures were examined and utilized for further purpose. For the synthesis of silver Nanoparticles, the streptomyces (actinomycete) culture was grown in 250 ml Erlenmayer flasks containing 100ml of Starch casein nitrate agar medium. Then the culture was grown with continuous shaking on rotary shaker at 28^{o}C for 96hrs (120 rpm). After 96hrs fermentation, mycelia

(cells) were separated from the culture broth by using what Mann filter no.1 at sterile conditions**.** Then the mycelium was washed thrice with sterile distilled water. The harvested mycelial mass was (10g of wet mycelia), then re-suspended in 100ml sterile distilled water and allowed to grow for 3 days on shaker at 120 rpm. After 3 days the mycelia (cells) are separated from filtrate by using what Mann filter paper no.1. Then the 1mM aqueous AgNO3 was resuspended into 100ml of filtrate of Streptomyces species. The whole mixture was put into a shaker at 28^{o}C (120 rpm) and maintained in dark and light conditions. The bio-reduction of the AgNo3 ions in solution was monitored and measuring the UV-Vis Spectra of the solution. The bio-reduction of Ag^{+} in aqueous solution was monitored by measuring the UV-Visible spectrum of the reaction medium at different time interval and different nanometer (280-580). UV-Vis spectra were recorded at 24hrs, 48 hrs, 72 hrs and 96 hrs. This UV-Vis spectral analysis has been done by using a Perkin-Elmer Lamda-25 spectrophotometer.

The particle size analysis of *Streptomyces sp.* nanoparticles has been obtained by the dynamic light scattering technique of laser light using particle size analyzer (Nanophox, Germany). This study was undertaken to know the size and shape of the silver nanoparticles biosynthesized using sample of *Streptomyces sp,* and recovered the silver nanoparticles by ultra-centrifugation for characterization. Due to our interest to get much smaller particles, above solution was centrifuged at a rate of 10000 rpm for 15 minutes and collected the supernatant was air dried under hot air oven. The dried silver nanoparticles were subjected to SEM analysis. The images of nanoparticles were obtained in a Scanning Electron Microscope (Department of Nanoscience & Technology, Bharathiar University, Coimbatore). The dried silver nanoparticles were subjected to FTIR analysis. The chemical groups present in the nanoparticles have been studied using FTIR (Perkin Elmer, USA). The air dried nanoparticles were coated on XRD grid and analyzed for the formation of Ag nanoparticles by Philips X-Ray Diffract meter with Philips PW 1830 X-Ray Generator operated at a voltage of 40kV and a current of 30mA with Cu Kα1 radiation. The diffracted intensities were recorded from 10˚ to 80˚ of 2θ angles.

The antimicrobial activities of silver nanoparticles were investigated by disc diffusion method (Cruickshank 1968). Silver nanoparticles synthesized using sample of *Streptomyces sp.,* are tested for its potential antimicrobial activity against few human pathogens. The sterile disc was dipped in silver nanoparticle solution and placed in the agar plate in the following order Disc and Disc + silver nanoparticles and kept for incubation at 37°C ± 0.2 C for 24 hrs. To analyze the antimicrobial activity of the sample, the samples were subjected to Agar well Diffusion Techniques as described by (Agarry *et al*., 2005). Wells of 8 mm diameter were cut on sterile nutrient agar plates and swabbed with an overnight broth culture of the organism. Each well was loaded with 40µl the solutions in the following order: silver nitrate solution and silver nanoparticles of *Streptomyces sp.,* and incubated at 37°C ± 0.2 C. Antimicrobial activity in terms of zones of inhibition (mm) was recorded after 24 h of incubation. The antagonistic actions of silver nanoparticles of *Streptomyces* sp. were tested against test organisms in triplicates. Pure cultures of bacteria namely *Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Shiegella dysentriae* and *Salmonella typhi* and they cause most of the hospital infections. The evaluation of agar diffusion test was made on the basis of zone of inhibition of bacteria around the test samples.

Result and Discussion

The formation of silver nanoparticles in the solution of 1mM silver nitrate and sample of *Streptomyces Sp* was confirmed by change in the color to yellow after incubation with silver nitrate, while the controls retained the original color.

Nitrate reductase test was conforming the enzyme Nitrate reductase present in the Extracellular filtrate of the silver nanoparticles synthesized by *Streptomyces sp*.

The bioreduction of Ag^+ in the aqueous filtrate was monitored by periodic sampling of the reaction mixture at regular intervals by using UV-vis spectroscopy. The silver anoparticles exhibits dark brown color and this arises due to excitation of surface plasmon vibrations in the metal nanoparticles (Mulvaney et al., 1996). The fig.2 show the UV-Vis spectra recorded from the aqueous silver nitrate – filtrate of streptomyces species. A strong characteristic absorbance peak at around 450 nm was observed at different time intervals.

Particle size analysis shows that, when scanning from 1 nm, the particles count is very low and it's gradually increasing from highest value above100 nm this is because of agglomeration of particles. So this indicates that the maximum nanoparticles in the range of 100 nm and few particles are present above this range.

Scanning electron microscopic analyses of the silver nanoparticles reduced formof silver nitrate solution through bioreduction are clearly distinguishable owing to their size difference. It is clear from the SEM pictures that silver particles in the bio-reduced colloidal suspensions measured ~ 70nm in size, Fig. 4. The particles are spherical in shape, well defined, separated as much as possible. This may be due to the reduction in liquid solution and some chelating action also available in the solution. Due to this Silver particle nucleation is higher than the particle agglomeration.

X-Ray Diffraction studies of the sample is shown fig 4. XRD analysis shows three distinct diffraction peaks at 38.3˚, 44.2˚, 64.0˚ and can be indexed 2θ values of (111), (200), (220) crystalline planes of cubic Ag. The average grain size of the silver nanoparticles formed in the bioreduction process is determined using Scherr's formula, $d=(0.9\lambda*180^{\circ}) / \beta \cos\theta\pi$ and is estimated to be 75 nm. Fig. 5. shows the XRD pattern of the silver nanoparticles formed in our experiment.

FTIR spectral analysis showed array of absorbance bands in 600 cm^{-1} - 4000 cm^{-1}. The spectral bands were prominent at 1137 cm^{-1}(Alcohols, P=O), 1382 cm^{-1} (Alkanes, Nitro Compounds) 1655cm^{-1}(Amines) and 3483 cm^{-1}(Amines, Alcohols & Phenols) which were interpreted for the identification of the functional moieties in the air dried silver nanoparticles. Fig. 6. shows the FTIR spectrum of the silver nanoparticles.

The antimicrobial activity was carried out using five different strains. Zone of Inhibition in the plate showed that silver nanoparticles synthesized using sample of *Streptomyces sp.*, have the antibacterial activity against test pathogens namely *Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Shiegella dysentriae, Salmonella typhi* and *Klebsiella pneumonia*. On comparison with the antibiotics and *Streptomyces sp.* Synthesized silver nanoparticles out performed in the bactericidal effect (Fig 7 and 8).

The present work is supposed to be the first extensive work in antimicrobial activity of

Streptomyces sp synthesized silver nanoparticles. To test the antimicrobial activity of silver nitrate and silver nanoparticles synthesized by *Streptomyces sp.* the inhibition zone formation around the disc was noted and measured the diameter of that inhibition zone range.

The antimicrobial activity of silver nitrate and silver nanoparticles synthesized by *Streptomyces sp* were confined against disease causing microorganism namely *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, and *Salmonella typhi* through the inhibition zone formation range. The antimicrobial activities of silver nanoparticles synthesized by *Streptomyces sp.* were higher than silver nitrate for disease causing micro organisms namely *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Shiegella dysentriae* and *Salmonella typhi* and it was confined through their inhibition zone range. (Fig 7).

For testing the antimicrobial activity of silver nitrate and silver nanoparticles synthesized by *Streptomyces sp.* , the inhibition zone formation around the well was noted and measured the diameter of that inhibition zone range. The antimicrobial activity of silver nitrate and silver nanoparticles synthesized by *Candida albicans* were confined against disease causing microorganism namely *Staphylococcus aureus*, *Escherichia coli*, *Shiegella dysentriae*, *Salmonella typhi* and *Klebsiella pneumonia* through the inhibition zone formation. The antimicrobial activities of silver nanoparticles synthesized by *Streptomyces sp.* were more than 50% higher antimicrobial activity than silver nitrate for disease causing micro organisms namely *Staphylococcus aureus*, *Escherichia coli*, *Shiegella dysentriae*, *Salmonella typhi* and *Klebsiella pneumonia* than silver nitrate and it was confined through their inhibition zone range.

Tabel.1 Disc Diffusion Method

Name of the species	*Staphylococcus aureus*	*Escherichia coli*	*Proteus vulgaris*	*Salmonella typhi*
Silver nitrate	8 mm	18 mm	7 mm	7 mm
Silver nanoparticles	20 mm	22 mm	17 mm	16 mm

Tabel.2 Zone of Inhibition range by Well Diffusion method

Compound	*Staphylococcus aureus*	*Escherichia coli*	*Klebsiella pneumonia*	*Shiegella dysentriae*	*Salmonella typhi*
Silver nitrate (Control)	14 mm	16 mm	9 mm	10 mm	10 mm
Silver nanoparticles	23 mm	20 mm	15 mm	16 mm	16 mm

Fig.1 Biosynthesis of silver nanoparticles using Streptomyces sp

Fig.2 Nitrate reductase positive results using in the extra cellular filterate of the silver nanoparticles synthesized by Streptomyces sp

Fig.3 Graph showing the synthesized silver nanoparticles in different time intervals

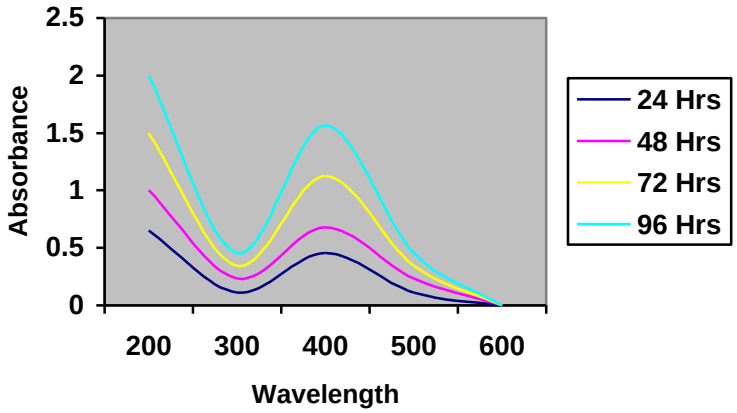


Fig.4 SEM analysis of silver nanoparticles synthesized by Strptomyces sp.

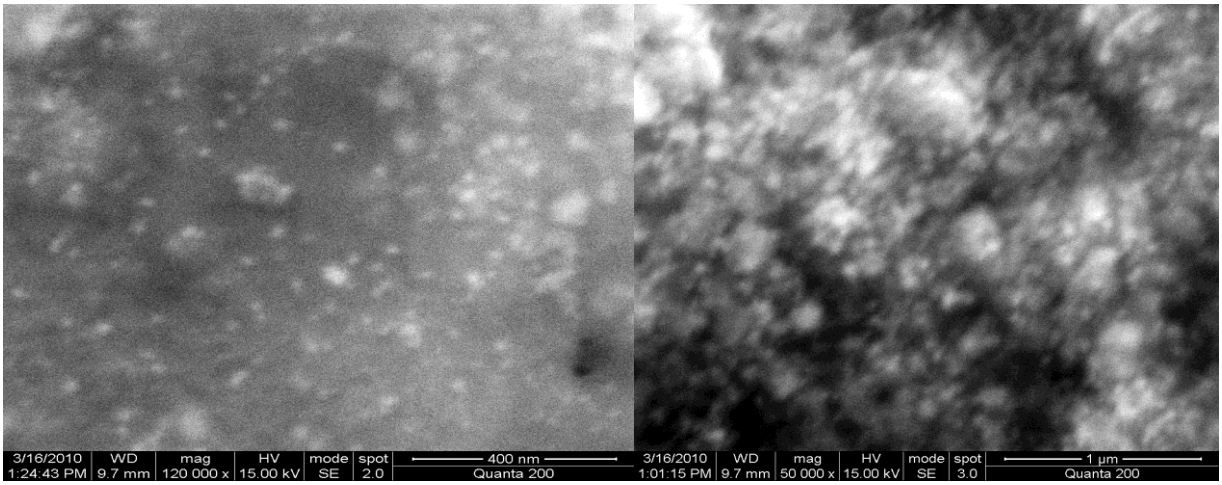


Fig.5 XRD analysis of silver nanoparticles synthesized by Strptomyces sp.

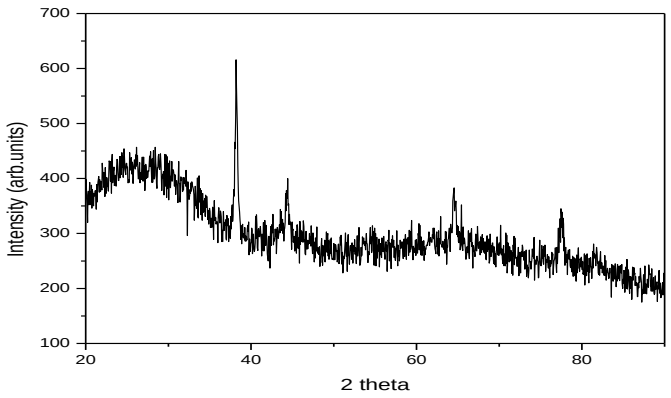


Fig.6 FTIR analysis of silver nanoparticles synthesized by Strptomyces sp.

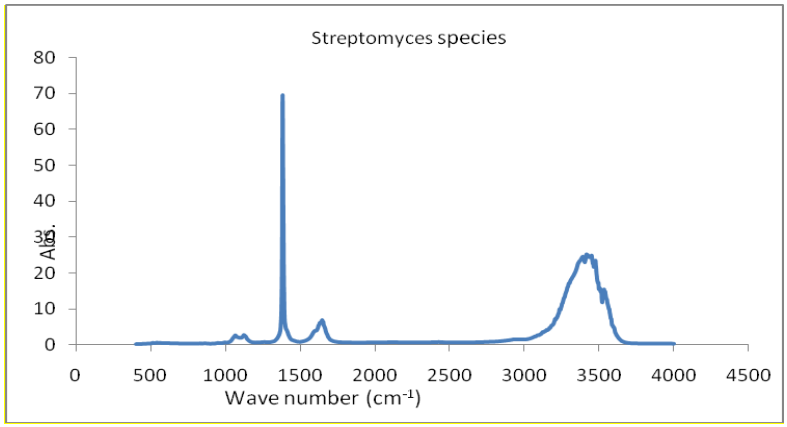


Fig 7: Antimicrobial activity of silver nanoparticles synthesized by Streptomyces sp by Disc diffusion method

Escherichia coli *Staphylococcus aureus*

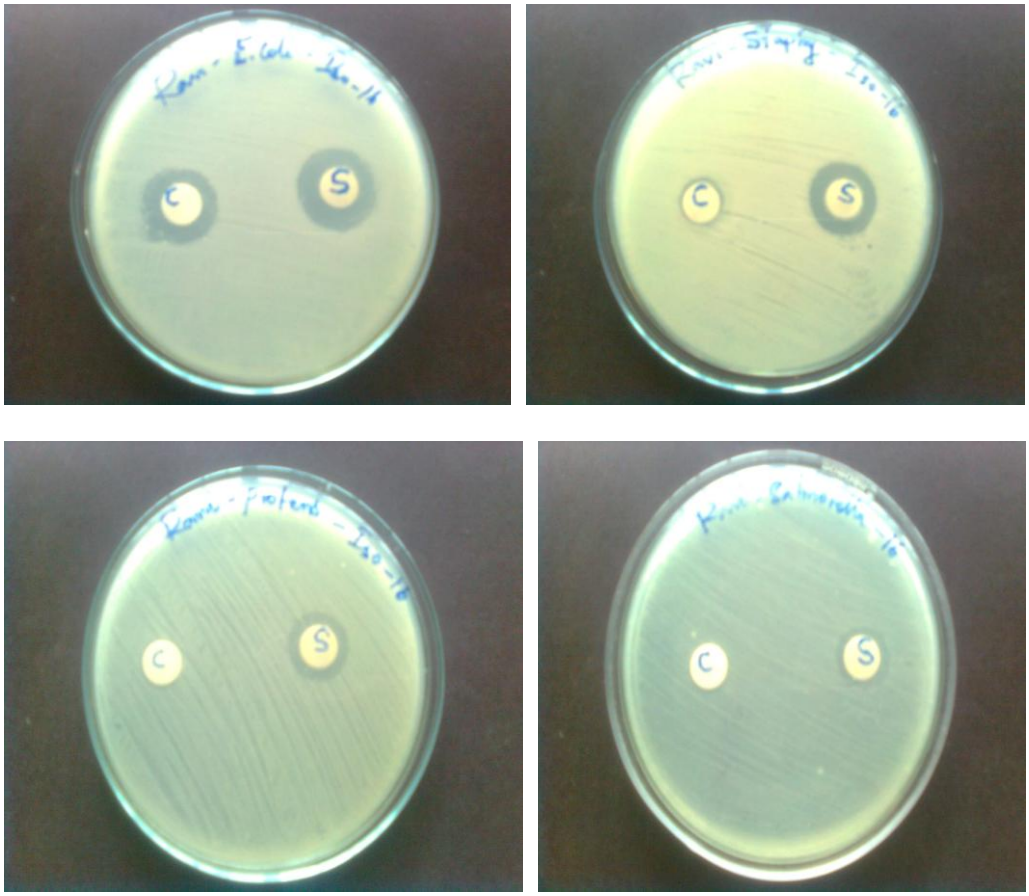
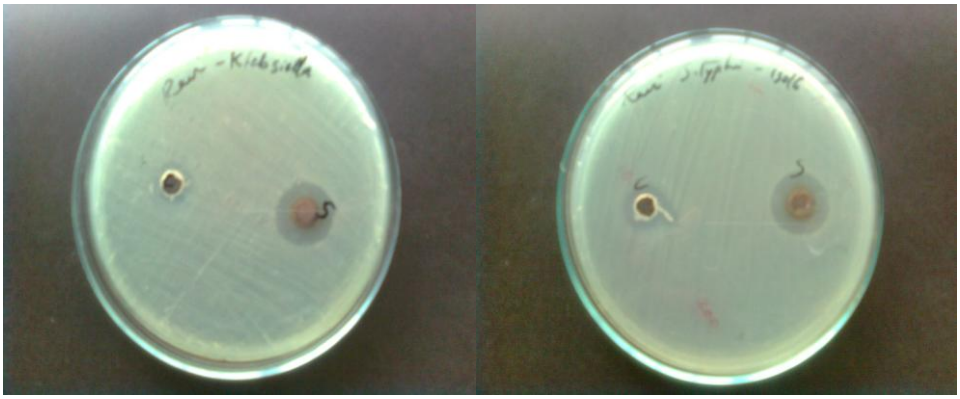
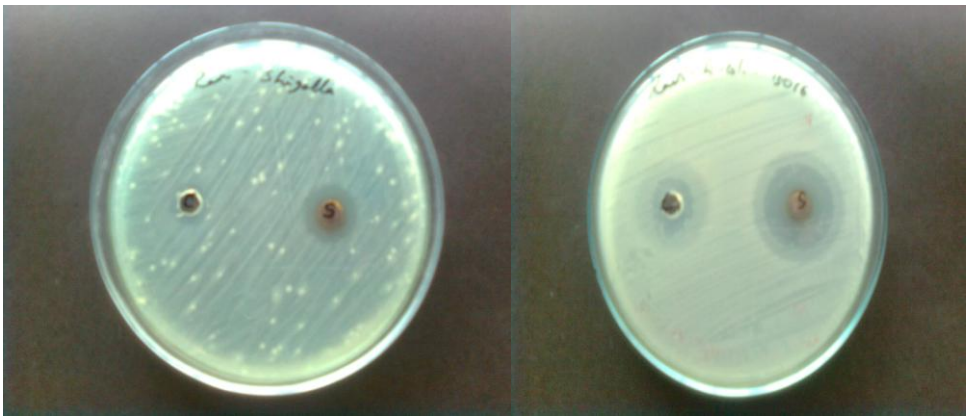


Fig.7 Antimicrobial activity of silver nanoparticles synthesized by Streptomyces sp by Well diffusion method

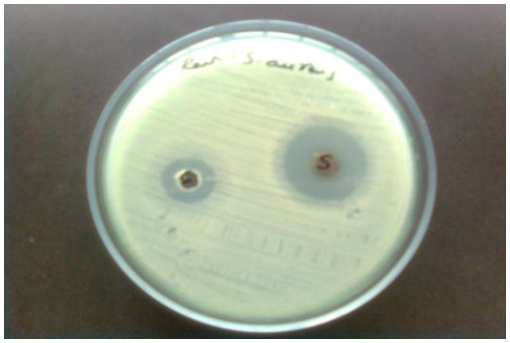
Klebsiella pneumonia *Salmonella typhi*



Shiegella dysentriae *Escherichia coli*



Staphylococcus aureus



Addition of *Streptomyces sp*, to 1mm solution of silver nitrate led to the appearance of dark brown color as resultant of formation of silver nanoparticles in the solution. The UV-Vis absorption spectrum recorded for the solution shows the characteristic surface plasmon resonance band for silver nanoparticles in the range of 70-100 nm. The pale dark brown appears immediately after the addition of the sample *Streptomyces sp*, and the reaction is completed in about 3hrs. This makes the investigation highly significant for rapid synthesis of silver nanoparticles. The SEM studies confirmed the formation of silver particles in the size range of 40-100 nm, a clear indication of the formation of silver nanoparticles. XRD analysis shows three distinct diffraction peaks at 38.3˚, 44.2˚, 64.0˚ and can be indexed 2θ values (111), (200), (220) crystalline planes of cubic Ag. The average grain size of the silver nanoparticles formed in the bioreduction process is determined using Scherr's formula, d=(0.9λ*180˚) / β cosθπ and is estimated to be 60 nm. FTIR spectral analysis showed array of absorbance bands in 600 cm^{-1} - 4000 cm^{-1}. Organic functional groups are available in the air dried silver nanoparticles. The spectral bands were prominent at at 1137 cm^{-1}(Alcohols, P=O), 1382 cm^{-1} (Alkanes, Nitro Compounds) 1655cm^{-1}(Amines) and 3483 cm^{-1}(Amines, Alcohols & Phenols). This organic group presence is due to Silver Particles reduction through biological sources. Ultra pure Ag particle can be prepared by removing the functional groups through chemical

modification in future studies. We have found that the silver nanoparticles synthesized in our study effectively inhibited the growth and multiplication of pathogens like *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Shiegella dysentriae* and *Salmonella typhi*. On comparison with the silver nitrate, and the silver nanoparticles outperformed in the bactericidal effect.

References

Agarry O O Olaleye M T Bello Michael C O 2005.Comparative antimicrobial activities of Aloevera gel and leaf. Afr.J. Biotechnolol 4 : 1413 -1414.

Ahmad,A., Senapati,S., Khan ,M.I., Kumar, R., Sastry, M. 2003. Extracellular biosynthesis of monodisperse gold nanoparticles by a novel extremophilic actinomycete, *Thermomonospora* sp. Langmuir 19: 3550–3553.

Cruickshank, R. 1968. Medical microbiology: A guide to diagnosis and control of infection, 11th edn. E & S Livingstone Ltd. Edinburgh and London. p.896.

Kathireswari et al. 2014. Plant leaf mediated synthesis of silver nanoparticles using *Phyllanthus niruri* and its antimicrobial activity against multi drug resistant human pathogens. Int.J.Curr.Microbiol.App.Sci (2014) 3: 960 – 968.

Lazzarini A, Cavaletti L, Toppo G, Marinelli F. 2000. Rare genera of actinomycetes as potential producers of new antibiotics. Ant. Leeuw., 78, pp. 399–405.

Mann, S., 1993. Molecular tectonics in biomineralization and biomimetic materials chemistry.Nature, 365:499-505.

Mulvaney, P., 1996. Surface plasmon spectroscopy of nano sized metal particles.Langmuir,1:788-800.

Sastry M, Ahmad A, Khan MI, Kumar R 2003. Biosynthesis of metal nanoparticles using fungi and actinomycete. Curr Sci 85:162–170

Schuller, M., Laude, J., Bodewaldt, H., Hamm, G., Korting, H.C. 2004. Toxicity and antimicrobial activity of a hydrocolloid dressing containing silver particles in an ex vivo model of cutaneous infection. Skin Pharm. Physiology.17: 31.