

**SCREENING, ISOLATION, IDENTIFICATION,
CHARACTERISATION AND APPLICATIONS OF SILVER
NANOPARTICLES SYNTHESIZED FROM MARINE
ACTINOMYCETES (STREPTOMYCES GRIESEORUBENS)**

N. Vidhyashree* and Dr. S. Yamini Sudha Lakshmi

Department of Medical Biochemistry, University of Madras, Taramani Campus, Chennai –
600113.

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***Correspondence for
Author**

N. Vidhyashree

Department of Medical
Biochemistry, University
of Madras, Taramani
Campus, Chennai –
600113.

ABSTRACT

Crystalline Silver Nano particles have been found to have diagnostic and therapeutic applications in medical field. In this study, the silver nanoparticles were synthesized biologically using silver as reducing agent. The extract was used from marine Actinomycetes for the study of screening, identification, characterization, antimicrobial, free radical scavenging and anti-cancer activities. Screening was done for anti-microbial activity. With 16SrRNA sequencing, the organism was identified as *Streptomyces grieseorubens*. The formation of silver nanoparticles were indicated by the colour change from colourless to reddish brown, characterized by SEM, TEM revealing the size of nanoparticle to be 50nm having hexagon shape. XRD and FTIR showing the crystalline nature and the functional groups (OH, C-C,

NH) present in the nanoparticle. The antimicrobial study was made against *B.subtilis*, *E.coli*, *S.aureus*, and *Proteus vulgaris*. *S.aureus*, showed the maximum zone of inhibition around 15mm. These nanoparticles were found to have a significant free radical scavenging activity and a potent cytotoxic activity against MCF7 cell line *invitro*.

KEY WORDS: Cytotoxic, Antibacterial, Free radical and Nanoparticles.

INTRODUCTION

Actinomycetes: Actinomycetes are economically and biotechnologically most valuable prokaryotes. They are responsible for the production of about a half of the discovered

bioactive secondary metabolites, notably antibiotics (Berdy 2005, Strohl 2004), antitumor compounds (Cragg *et al.*, 2005), immunosuppressive agents (Mann *et al.*, 2001) and enzymes (Oldfield *et al.*, 1998). Soil microorganisms provide an excellent resource for the isolation and identification of therapeutically important products. Actinomycetes are gram-positive bacteria with high guanine + cytosine content of over 55% in their DNA, which have been recognized as sources of several secondary metabolites, antibiotics, and bioactive compounds that affect microbial growth (Ishida *et al.*, 1965). The term actinomycete is a common name that is most appropriately applied to bacteria belonging to the Order Actinomycetales. This Order falls within the recently redefined Class Actinobacteria (Stackebrandt *et al.*, 1997) and, as a result, all Actinobacteria are not actinomycetes. This is of particular relevance to discussions of marine actinomycetes, as Actinobacteria are frequently observed when culture-independent techniques are applied to studies of marine bacterioplankton (Giovannoni and Ulrich 2005).

Taxonomic classification

DOMAIN : Bacteria

PHYLUM : Actinobacteria

CLASS : Actinobacteria

ORDER : Actinomycetales

FAMILY : Actinomycetaceae

Actinomycetes, characterized by a complex life cycle, are filamentous Gram-positive bacteria belonging to the phylum *Actinobacteria* that represents one of the largest taxonomic units among the 18 major lineages currently recognized within the domain Bacteria (Ventura, *et al.*, 2007). Actinomycetes have filamentous nature, branching pattern, and conidia formation, which are similar to those of fungi. For this reason, they are also known as ray fungi (Wang, *et al.*, 1999). Actinomycetes produce branching mycelium which may be of two types, viz., substrate mycelium and aerial mycelium. *Streptomyces* are the dominant of all actinomycetes (Okami, *et al.*, 1972).

Actinobacteria are widely distributed in terrestrial and aquatic ecosystems, especially in soil, where they play a crucial role in the recycling of refractory biomaterials by decomposing complex mixtures of polymers in dead plant, animal and fungal materials. They are important in soil biodegradation and humus formation by the recycling of nutrients associated with recalcitrant polymers such as keratin, lignocelluloses and chitin (Goodfellow, *et al.*, 1983;

McCarthy, *et al.*,1992; Stach, *et al.*,2004) and produce several volatile substances like geosmin responsible of the characteristic “wet earth odor” (Wilkins *et al.*,1996). They are efficient producers of new secondary metabolites that show a range of biological activities including antibacterial, antifungal, anticancer, insecticidal and enzyme inhibition (Ayuso-Sacido 2005). They also exhibit diverse physiological and metabolic properties, such as the production of extracellular enzymes (Schrempf *et al.*, 2001).

MATERIALS AND METHODS

Sample Collection: Marine sediments were collected from marina the coast of Tamilnadu at 10 m length and 5 m depth with sterile spatula in sterilize polythene bags. The samples were closed tightly transported to the laboratory aseptically and stored in the refrigerator at 4°C until further use. (Zarina *et al.*, 2014).

Isolation of Actinomycetes : Collected soil samples were treated with 1% calcium carbonate and air dried for 3 to 4 days under in vitro lab condition to increase the number of actinomycetes propagules in the samples (Zarina *et al.*, 2014).

Soil Dilution Technique

Isolation of actinomycetes was performed by soil dilution plate technique (Ellaiah *et al.*, 1996). The pretreated soil sample was serially diluted in a sterile sea water from 10⁻¹ to 10⁻¹⁰ dilutions (Sourav *et al.*, 2010). The medium used for the isolation and cultivation of marine actinomycetes was starch casein agar medium contains (g/L) starch 10g, casein 0.3g, KNO₃ 2g, NaCl 2g, K₂HPO₄ 2g, MgSO₄·7H₂O 0.05g, CaCO₃ 0.02g, FeSO₄·7H₂O, 0.01g and agar 18g (Kuster *et al.*, 1964). 200 ml of starch casein agar media in 500 ml flask were sterilized at 121°C for 20 min by autoclaving. The media was prepared by using 50% (v/v) sea water and 50% (v/v) distilled water. After autoclaving medium was plated in a petri dish under aseptic condition (Sahu *et al.*, 2004). Each test tube of diluted samples were vortexed vigorously for 15 minutes. From each suitable dilution, 0.1 ml was taken and spread evenly with sterile L-shaped glass rod over the surface of starch casein agar media and kept for incubation at 30°C for 7 days.

Appearance and growth of marine actinobacteria were monitored regularly. The colonies were recognized by their characteristic chalky to leathery appearance on SCA plates.

Anti-microbial activity for Screening

The antimicrobial activity was studied preliminary by cross streak method against bacteria and fungi. The test organisms used are *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Rhizoctonia solani* and *Candida albicans*. After the preliminary test of the isolates for their antimicrobial activities, the most active isolate was subjected to 16S rRNA sequencing to identify the genus and species.

16 srrna sequencing

Genomic DNA isolation and pcr analysis

Genomic DNA was extracted from overnight grown cultures of the selected bacterial isolates using QIAGEN DNA isolation kit (Qiagen), suspended in 100µl of elution buffer (10mM/L Tris-HCl, pH 8.5) and quantified by measuring OD at 260nm. PCR amplification was performed using a 20µl reaction mixture containing 100ng of template DNA, 20µmol of 16S rRNA primers, 200µM of dNTPs, 1.5mM of MgCl₂, 1U of Taq DNA polymerase (MBI Fermentas) and 2µL of 10x Taq polymerase buffer. The sequences of 16S rRNA primers used were as follows.

FP 5'-AGAGTTTGATCCTGGCTCAG-3'

RP 5'-ACGGCTACCTTGTTACGACTT -3'

Amplification was carried out with an initial denaturation at 95°C for 5min followed by 35 cycles of denaturation at 94°C for 45sec, annealing at 56°C for 45sec, extension at 72°C for 1min and final extension at 72°C for 5min using a thermocycler (iCycler; Bio-Rad Laboratories, CA). PCR products were analyzed on 1% agarose gel for 16S rRNA amplicons in 1x TBE buffer at 100V.

Cloning and sequence analysis of pcr products

The 16S rRNA amplified fragments were purified using the QIA quick gel extraction kit (Qiagen, Valencia, CA) from the agarose gel and sequenced using automated DNA sequencer (Model 3100, Applied Biosystems, USA). The sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) software (<http://www.ncbi.nlm.nih.gov/blast>) against the 16S ribosomal RNA sequence database and submitted in Gene Bank.

Phylogenetic analysis: The sequences of these 16SrRNA genes were compared against the sequences available from Gene Bank using the BLASTN program (Altschul *et al.*, 1990) and

were aligned using CLUSTAL W software (Thompson *et al.*, 1994). Distances were calculated according to Kimura's two-parameter correction (Kimura, 1980). Phylogenetic trees were constructed using the neighbour-joining method (Saitou and Nei 1987).

Bootstrap analysis was done based on 1000 replications. The MEGA5 package (Kumar *et al.*, 2004) was used for all analyses.

Preparation of culture media: The isolate *Streptomyces grieseorubens* was inoculated into 250 ml of ISP-2 broth culture, incubated at 30°C for 7 days under shake flask condition. After the incubation period was complete, the culture was centrifuged at 5000rpm for 30min and the supernatant was used for the biosynthesis of AgNPs (Priyanka 2011).

Anti-bacterial assays (well diffusion method)

Antibacterial activity was assayed by using the agar well diffusion test technique. Nutrient agar medium (NA) was prepared, the pH of the medium was maintained at 7.4 and then it was sterilized by autoclaving at 121°C and 15 lbs pressure for 15 min. 20 ml of the sterilized media was poured into sterilized petri dishes and allowed to solidify at room temperature. A sterile cotton swab is used for spreading each test microorganisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris* and *Rhizoctonia solani*, from the 24 h inoculated broth evenly on the NA plates and left for a few minutes to allow complete absorption of the inoculums. In each of these plates 5-mm diameter wells were made at the centre using an appropriate size sterilized cork borer. Different concentrations of sample were added to the respective wells on the NA agar plates. Concentration ranges from 25, 50, 75 and 100 microlitre respectively, were placed in the wells and allowed to diffuse at room temperature for 30 min. No AgNPs was added in the control plate. The AgNPs loaded plates were kept in incubation at 37°C for 24 h. After incubation, a clear inhibition zone around the wells indicated the presence of antimicrobial activity (Nathan 1978).

Free radical scavenging activity (DPPH assay)

The ability of the AgNPs to annihilate the DPPH radical (1,1-diphenyl-2-picrylhydrazyl) was investigated. Stock solution of sample was prepared to the concentration of 1mg/ml. 20,40,60,80,100,120,140,160,180 μ g of each sample were added to DPPH (0.1%). The reaction mixture was incubated for 30 min at room temperature and the absorbance (A) was recorded at 517 nm. The experiment was repeated for three times. BHT (Butylated Hydroxy Toluene) was used as standard control. The annihilation activity of free radicals was

calculated as % inhibition according to the following formula; % Radical scavenging activity (%RSA) = $[(A \text{ of control} - A \text{ of sample}) / (A \text{ of control})] \times 100$.

A of control is the absorbance of BHT radical + ethanol; A of sample is the absorbance of DPPH radical + AgNPs / standard.

Cytotoxicity assay on cancer cell lines

Chemicals and reagents

1. MTT (3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay.
2. Trypan blue.
3. RPMI medium for culturing of cells
4. Fetal Bovine Serum

Cell culture

MCF 7/ HepG 2 cells were cultured in Rose well Park Memorial Institute medium (RPMI), supplemented with 10% fetal bovine serum, penicillin/streptomycin (250 µg/ml), gentamycin (100µg/ml) and amphotericin B (1mg/ml). All cell cultures were maintained at 37°C in a humidified atmosphere of 5% CO₂. Cells were allowed to grow to confluence over 24 h before use.

Evaluation of *in vitro* cytotoxic activity of the silver nanoparticles on cell lines

MTT assay was performed to determine the cytotoxic property of synthesized AgNPs against HepG-2, MCF7, HT29 and Vero cell lines. Cell lines were seeded in 96-well tissue culture plates. Stock solutions of AgNPs (5 mg/ml) were prepared in sterile distilled water and diluted to the required concentrations (10, 5, 2.5, 1.25, 0.625, 0.312, 0.156 mg/ml) using the cell culture medium. Appropriate concentrations of Ag-NP stock solution were added to the cultures to obtain respective concentration of Ag-NP and incubated for 48 hrs at 37°C. Nontreated cells were used as control. Incubated cultured cell was then subjected to MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) colorimetric assay [J. Saraniya Devi and B. Valentin Bhimba, 2012]. The tetrazolium salt 3-[4, 5-dimethylthiazol-2-yl] - 2, 5-diphenyltetrazolium bromide (MTT) is used to determine cell viability in assays of cell proliferation and cytotoxicity. MTT is reduced in metabolically active cells to yield an insoluble purple formazon product. Cells were harvested from maintenance cultures in the exponential phase and counted by a hemocytometer using trypan blue solution. The cell suspensions were dispensed (100µl) in triplicate into 96-well culture

plates at optimized concentrations of 1×10^5 /well for each cell lines, after a 24- hr recovery period. Assay plates were read using a spectrophotometer at 520 nm. The spectrophotometrical absorbance of the samples was measured using a microplate (ELISA) reader. The cytotoxicity data was standardized by determining absorbance and calculating the correspondent AgNP concentrations. Data generated were used to plot a dose-response curve of which the concentration of extract required to kill 50% of cell population (IC_{50}) was determined.

Cell viability (%) = Mean OD/ control OD \times 100 (Saraniya Devi, et al., 2012).

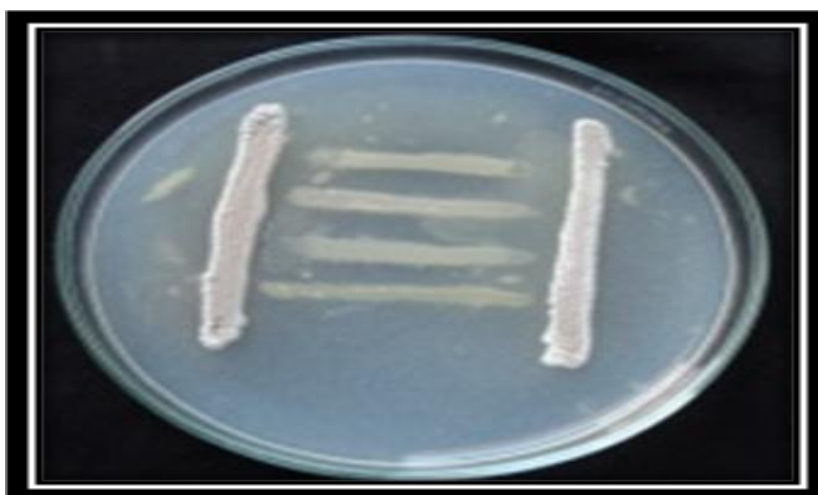


Fig.1 Antibacterial activity of *Streptomyces griseorubens* against 1 – *Bacillus subtilis* 2 – *Staphylococcus aureus* 3 – *Escherichia coli* 4 – *Proteus vulgaris*.

Anti-fungal activity

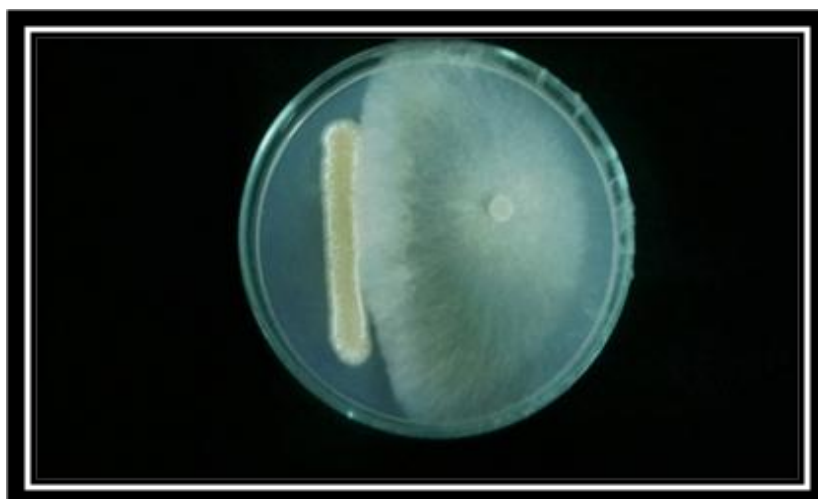


Fig.2 Antifungal activity of *Streptomyces griseorubens* against *Candida albicans*.

Species Identification

According to Bergey's manual of Determinative bacteriology, Ninth edition (2000) and the Laboratory Manual for Identification of Actinomycetes, the organism was identified as *Streptomyces grieseorubens*.

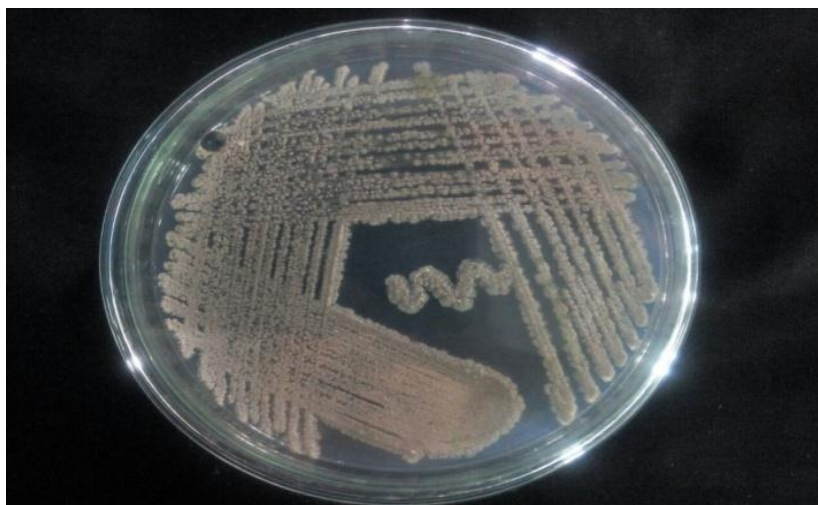


Fig.3 Marine actinomycetes streak plate culture

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GGCTCAGGACGAACGCTGGCGGCTGCTTAACACATGCAAGTCGAACGATGAACCACTTCGGTGGGGATT
AGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGACAAGCCCTGGAAACGGGGTC
TAATACCGGATACTGACCCGCTTGGGCATCCAAGCGGTTTCGAAAGCTCCGGCGGTGCAGGATGAGCCCGC
GGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAGGCGACGACGGGTAGCCGGCTGAGAGGGCGAC
CGGCCACACTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGG
GCGAAAGCCTGATGCAGCGACGCCGCTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGG
AAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGTAATACG
TAGGGCGGAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGA
AAGCCCGGGCTTAACCCGGGCTGTCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAAT
TCCTGGTGAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCGA
TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCTGGTAGTCACGCGCGTAAAC
GGTGGGCACTAGGTGTGGGCGACATTCACGTCGTCCGTGCCGAGCTAACGCATTAAGTGCCCCGCTG
GGGAGTACGGCCGCAAGGCTAAACTCAAAGGAATTGACGGGGGCCGACACAAGCGGCGGAGCATGTGGC
TTAATTCGACGCAACGCGAAGAACCTACCAAGGCTTGACATACACGGAAGCATCAGAGATGGTGCCC
CCCTTGTTGGTGGTGATACAGGTGGTGCATGGCTGTCGTGAGCTCGTGTGAGATGTTGGGTTAAGTCC
CGCAACGAGCGCAACCCCTTGTCCTGTTGCCAGCAGGCCCTTGTTGGTGTGGGACTCACGGGAGACCG
CCGGGGTCAACTCGGAGGAAGTGGGGACGACGTCAAGTCATCATGCCCTTATGTCTTGGGCTGCACAC
GTGCTACAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAA
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Fig.4 16S rRNA SEQUENCING (*Streptomyces grieseorubens*)

DESCRIPTIONS

Description	Max Score	Total score	Query Cover	E value	Ident	Accession
Streptomyces griseorubens strain NBRC 12780 16S ribosomal RNA gene, partial Sequence	2292	2292	99%	0.0	100%	NR_041066.1
Streptomyces variabilis strain NRRL B-3984 16S ribosomal RNA gene, partial sequence	2287	2287	100%	0.0	99%	NR_043840.1
Streptomyces griseoincarnatus strain LMG 19316 16S ribosomal RNA gene, complete Sequence	2274	2274	99%	0.0	99%	NR_042290.1
Streptomyces griseoincarnatus strain NBRC 12871 16S ribosomal RNA gene, partial Sequence	2272	2272	99%	0.0	99%	NR_112312.1
Streptomyces griseoflavus strain LMG 19344 16S ribosomal RNA gene, complete Sequence	2272	2272	99%	0.0	99%	NR_042291.1
Streptomyces erythrogriseus strain LMG 19406 16S ribosomal RNA gene, complete Sequence	2270	2270	99%	0.0	99%	NR_042294.1
Streptomyces aureofaciens strain IMET 43577 16S ribosomal RNA gene, partial Sequence	2268	2268	100%	0.0	99%	NR_115193.1
Streptomyces althoticus strain CSSP544 16S ribosomal RNA gene, partial sequence	2265	2265	100%	0.0	99%	NR_115392.1
Streptomyces griseoflavus strain NBRC 13044 16S ribosomal RNA gene, partial sequence	2257	2257	98%	0.0	99%	NR_112349.1
Streptomyces lateritius strain CSSP722 16S ribosomal RNA gene, partial sequence	2255	2255	98%	0.0	99%	NR_115438.1
Streptomyces matensis strain NRRL B-2576 16S ribosomal RNA gene, partial sequence	2254	2254	100%	0.0	99%	NR_116076.1
Streptomyces labedae strain CSSP735 16S ribosomal RNA gene, partial sequence	2254	2254	98%	0.0	99%	NR_115446.1
Streptomyces viridochromogenes strain NRRL B-1511 16S ribosomal RNA gene, partial sequence	2246	2246	100%	0.0	99%	NR_043843.1
Streptomyces labedae strain NBRC 15864 16S ribosomal RNA gene, partial sequence	2246	2246	98%	0.0	99%	NR_041192.1
Streptomyces griseoincarnatus strain CSSP407 16S ribosomal RNA gene, partial sequence	2244	2244	98%	0.0	99%	NR_115369.1
Streptomyces albogriseolus strain NBRC 3413 16S ribosomal RNA gene, partial sequence	2242	2242	99%	0.0	99%	NR_112487.1
Streptomyces viridodiataticus strain NBRC 13106 16S ribosomal RNA gene, partial sequence	2241	2241	99%	0.0	99%	NR_112371.1
Streptomyces tendae strain ATCC 19812 16S ribosomal RNA gene, complete sequence	2241	2241	100%	0.0	99%	NR_025871.1
Streptomyces viridochromogenes strain NBRC 3113 16S ribosomal RNA gene, partial sequence	2239	2239	99%	0.0	99%	NR_112482.1

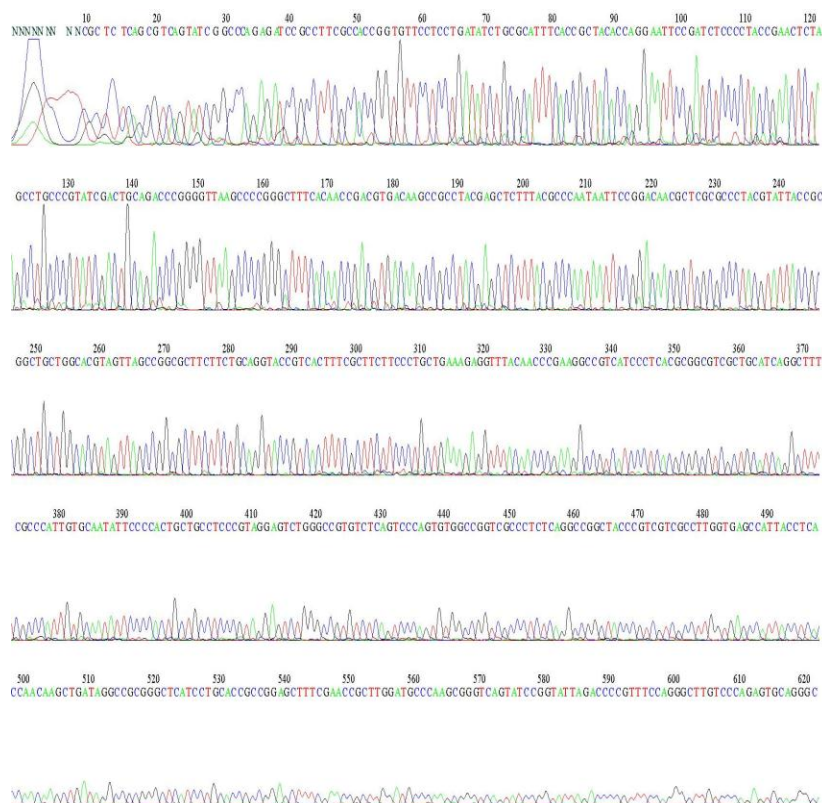


Fig.5 The BLAST Sequence

16S rRNA gene amplification and DNA sequencing in figure.4 explained that the 16S rRNA gene was amplified the sequence and revealed that the identified species was *Streptomyces griseorubens* from genomic DNA obtained from actinomycetes cultures by PCR. The amplification products were examined by agarose gel electrophoresis and purified by using a QIA quick PCR clean up kit. This work is similar to the work done by (Fredimoses, 2010) in 16S rRNA with the forward primer-F243 (5'-GGATGAGCCCGCGGCCTA-3') and reverse primer R513GC (5'-CGGCCGCGGCTGCTGGCACGTA-3'). The complete 16S rRNA gene was sequenced by using an ABI 377 automated DNA. This work similar to the work done by (Zotchev *et al.*, 2011), where the most comprehensive survey of the secondary metabolite biosynthesis potential for *marine actinomycetes* has been accomplished for the *Salinispora* spp. based on a thorough analysis of their genome sequences and 16S rRNA.

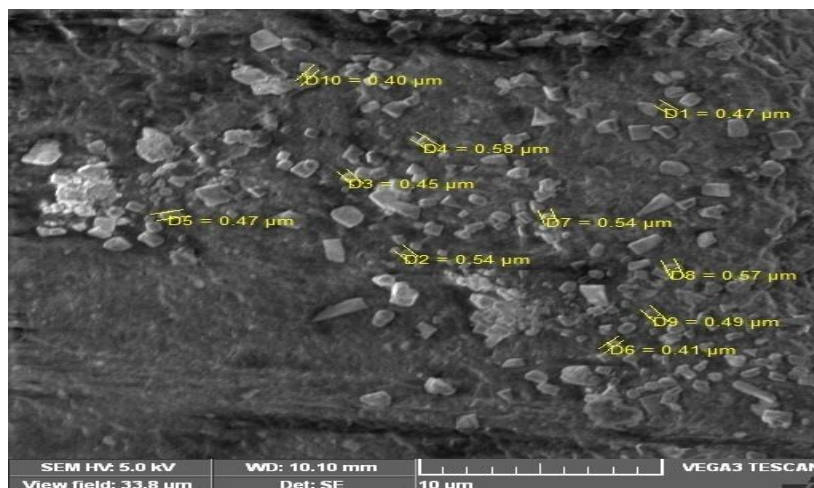
CHARACTERISATION**a) SEM**

Fig.6 SEM image of biosynthesized silver nanoparticles of isolated marine actinomycetes - *Streptomyces griseorubens*.

Scanning electron microscopy revealed the surface morphology of the nanomaterials, their shape, and size. Shape of the silver nanoparticles were found to be spherical. Some of the particles agglomerated and formed as a cluster.

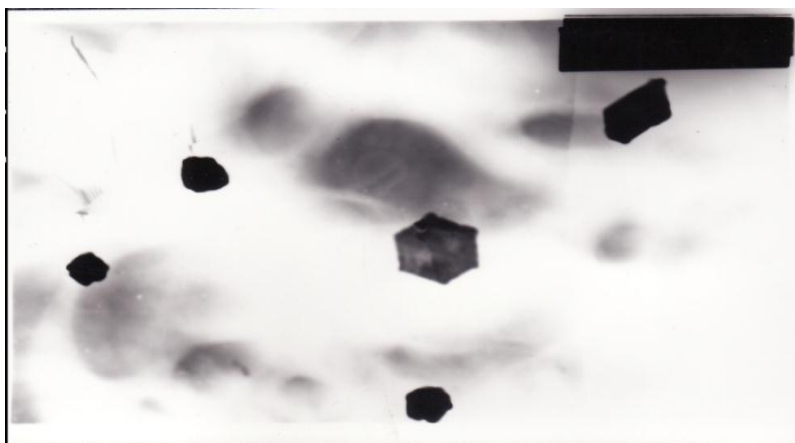
b) TEM

Fig.7 TEM image of biosynthesized silver nanoparticles of isolated marine actinomycetes - *Streptomyces griseorubens*.

The size of the silver nanoparticle was found to be around 50nm and the shape obtained is hexagon which was comparable with the results of SEM.

The present study reveals that the biosynthesized silver nanoparticles from the isolated *Streptomyces griseorubens* undergone SEM and TEM analysis found to be more or less in their uniform distribution and shape size of the silver nanoparticle which has been identified as hexagon and its size around 50nm which was comparable with the results of SEM. This work shows an effective result when compared to the similar work done by (Thiagarajan Hemalatha *et al.*, 2014) showed spherical nanoparticles are around 40-60 nm in size in the SEM and TEM characterisation of Hydroxyapatite-Coated Selenium Nanoparticles.

C) XRD

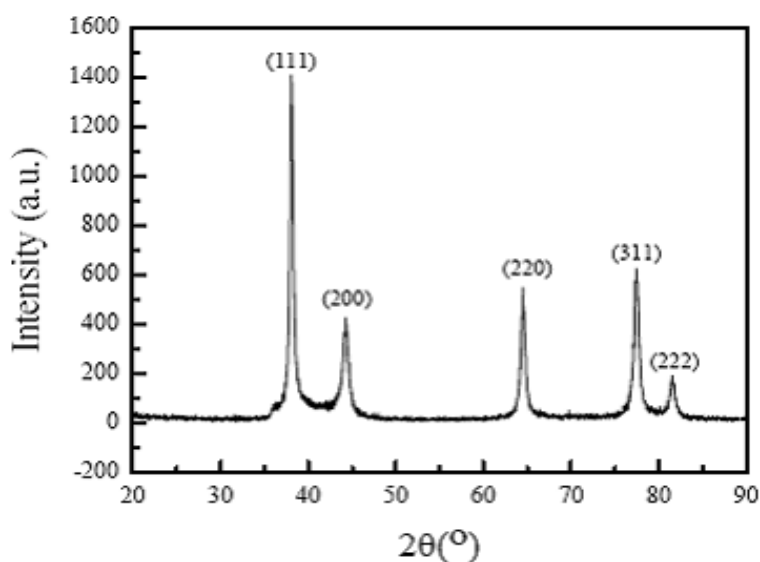


Fig. 8 X- Ray Diffraction of biologically synthesized silver nanoparticles from isolated marine actinomycetes - *Streptomyces griseorubens*.

2 θ values: 37.567, 44.632, 65.145, 78.523, 82.492

Plane values: 111,200,220,311,222.

XRD shows (fig:8) the crystalline nature of silver nanoparticles and the characteristic diffraction peaks at 2θ position. The characteristic X-ray Diffraction peaks of silver nanoparticles at 37.567, 44.632, 65.145, 78.523, 82.492 are seen in the XRD pattern.

This XRD characterization from the isolated marine actinomycetes - *Streptomyces griseorubens* in figure.8 represents the XRD patterns of the silver nanoparticles used. The average size of the synthesized silver nanoparticles calculated from XRD pattern. It indicates that the synthesized silver nanoparticles are crystalline in nature. The XRD pattern shows the

characteristic diffraction peaks at 2θ position. The characteristic X-ray Diffraction peaks of silver nanoparticles at 37.567, 44.632, 65.145, 78.523, 82.492 are seen in the XRD pattern. This work shows an effective result when compared to the similar work in which the patterns were recorded in the 2θ region of 10° - 90° in steps of 0.001° with a counting time of 1s in each step done by (Thiagarajan Hemalatha *et al.*, 2014).

c) FTIR

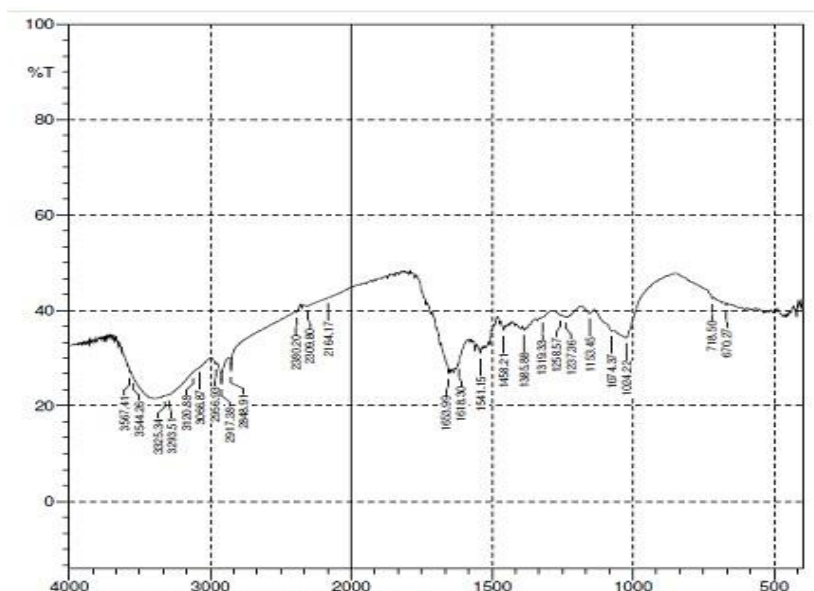


Fig. 9 FTIR analysis of biologically synthesized silver nanoparticles from isolated marine actinomyces - *Streptomyces griseorubens*.

The FTIR spectrum of silver nanoparticles from the isolated *Streptomyces griseorubens* in fig.9 shows a broad OH stretching absorption band between 3544.28 and 3587.41cm^{-1} and the C-H (aldehyde double peak) stretching between 3325.34 and 3293.51cm^{-1} . The maximum absorbance peak obtained was OH stretching band with the other functional groups such as amide protein, amide nitrogen representing different regions. The band at 3539.98 corresponded to an amide N-H band; band at 2083.96 corresponded to alkyne C-C and the band 1659.46 corresponded to amide C=O group. This evidence suggested the release of protein molecules that probably had a role in the formation and stabilization of AgNPs in aqueous solution.

Anti-Microbial Activity**Table: 1 Anti-bacterial activity**

Test organism	Zone of inhibition (mm)			
	250µg/ml	500µg/ml	750µg/ml	1000µg/ml
Bacillus subtilis	10	11	11	11.5
Escherichia coli	10	12	12	14
Staphylococcus aureus	11	13	14	15
Proteus vulgaris	11	12	12	13

Antibacterial activity(table-1) was done using the agar well diffusion method. *Streptomyces griseorubens* inhibits the microorganisms *Bacillus subtilis*, *Staphylococcus aureus* (ATCC1659), *Escherichia coli* (ATCC1879), *Proteus vulgaris* forming a zone of inhibition. However, Sondi and Salopek-Sondi reported the antimicrobial activity of silver nanoparticles was closely associated with the formation of ‘pits’ in the cell wall of bacteria, leading to increased membrane permeability and resulting in cell death [Sondi et al., 2004]. While Yamanaka et al. indicated that bactericidal actions of the silver ion are caused primarily by its interaction with the cytoplasm in the interior of the cell. The silver ion appears to penetrate through ion channels without causing damage to the cell membranes; it denatures the ribosome and suppresses the expression of enzymes and proteins essential to ATP production, which renders the disruption of the cell [Yamanaka et al., 2005]. In our present study bacterial cultures treated with silver nanoparticles showed increased conductance. This could be well attributed to the dissolution of the cellular contents in the culture broth, by the disruption of the cell membrane or as a result of complete destruction of the bacteria. It has also been hypothesized that Ag⁺ primarily affects the function of membrane-bound enzymes, which played vital role in the respiratory chain [Holt et al., 2005]. Silver has a greater affinity to react with sulfur- or phosphorus-containing biomolecules in the cell. Thus, sulfur-containing proteins in the membrane or inside the cells and phosphorus-containing elements like DNA are likely to be the preferential sites for silver nanoparticle binding [Bragg et al., 1974; McDonnell et al., 1999]. The possible mechanism of biosynthesis of nanoparticles by biological system was reductases and any other equivalent reductants [S.S. Shankar, et al., 2004].

Table:2 Free-radical scavenging activity

S.No	Concentration (μ g) of silver nanoparticles	Radical scavenging activity%	Radical scavenging activity%
		<i>Streptomyces griseorubens</i>	Standard (ButylatedHydroxyToluene)
1	20	8.79	18.75
2	40	13.7	38.52
3	60	19.45	49.23
4	80	25.89	51.56
5	100	29.22	54.29
6	120	32.68	62.46
7	140	38.41	68.92
8	160	47.80	71.52
9	180	50.42	77.48
10	200	54.33	86.16

Radical Scavenging Activity of silver nanoparticles synthesized from marine actinomycetes - *Streptomyces griseorubens*.

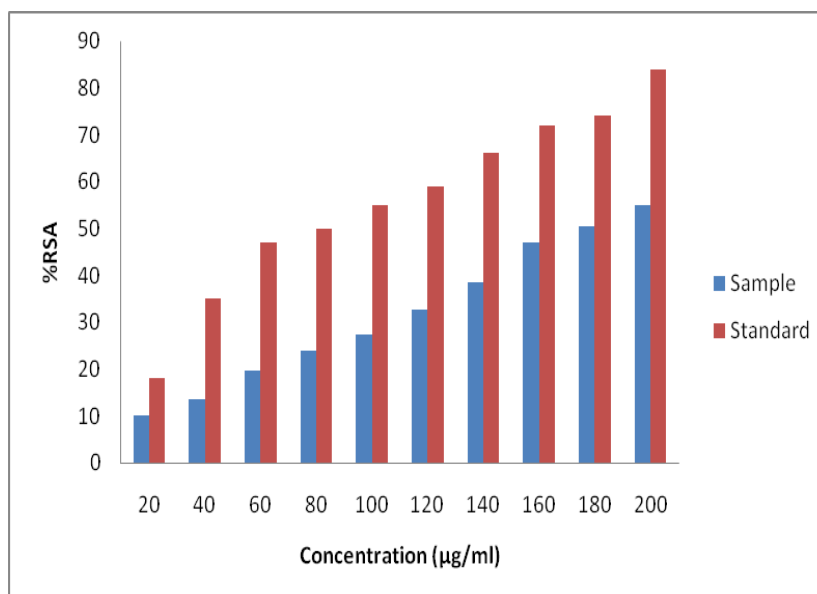


Fig.10 Graph indicating Radical Scavenging Activity of silver nanoparticles from marine actinomycetes -*Streptomyces griseorubens*.

The free radical scavenging activity of isolated marine actinomycetes - *Streptomyces griseorubens* increases with increase in silver nanoparticle concentration. This indicates that the cancer inducing cell death occurs and showed a maximum cell viability at 200 μ g..

This graph indicates the increase in radical scavenging activity with increase in concentration of silver nanoparticles. The standard used here is (Butylated Hydroxy Toluene).

Radical Scavenging Activity of silver nanoparticles using DPPH assay as shown in figure.10 was synthesized from *Streptomyces griseorubens* which had maximum cell viability at 200µg.

The data was considered significant when compared with the standard Butylated Hydroxy Toluene (BHT) used. The IC₅₀ value was calculated as 200µg/ml. This shows an effecting result while increasing the concentration of silver nanoparticles , the radical scavenging activity of *Streptomyces griseorubens* and the standard gets increased .The results of both the standard and the sample is compared in the graph. The standard used here is BHT (Butylated Hydroxy Toluene).

Table:3 Evaluation Of Cytotoxicity Of Agnps On Mcf7 Cell Lines Using Dpph Assay

S.NO	Concentration (µl)	Cell viability (%) of <i>Streptomyces griseorubens</i>
1	1	95.17
2	10	87.47
3	20	75.89
4	50	60.23
5	100	53.33

Evaluation of Cytotoxicity of AgNPs on MCF7 cell lines using DPPH assay

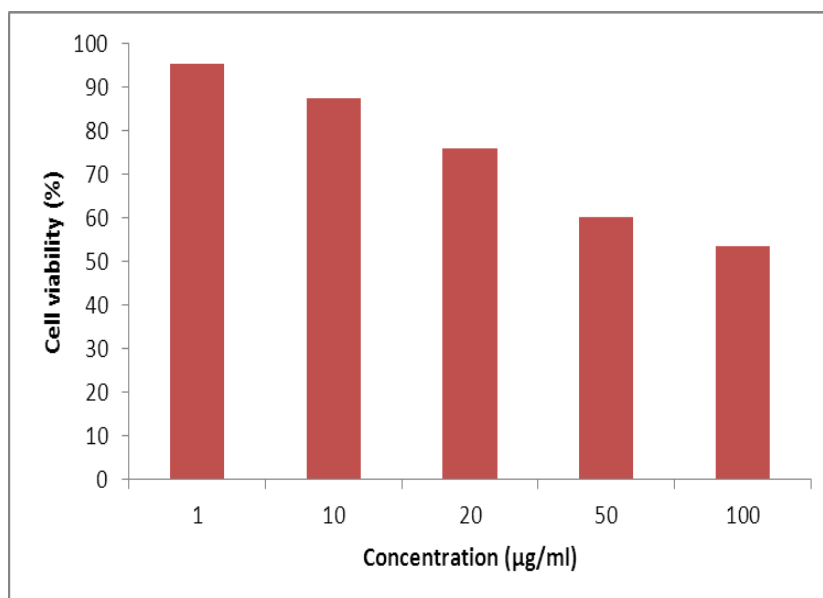


Fig.11 Evaluation of Cytotoxicity of Ag NPs on MCF7 cell lines using DPPH assay.

Figure.11 revealed the Evaluation of Cytotoxicity of AgNPs from isolated *Streptomyces grieseorubens* on MCF7 cell lines using MTT assay, it shows an anti-cancer activity of the biosynthesized AgNPs tested on MCF7 liver cell lines by MTT assay gave the efficient result with good activity at concentration 100µl using MCF7 liver cell line. A higher absorbance value in MTT assay denotes high rate of cell proliferation. Absorbance values that are lower depict that there is a decline in cell proliferation or the number of viable cells. This work is similar to the work in which the combination methods for screening marine actinomycetes producing potential compounds as anticancer in this study obtained the selected isolates of *marine actinomycetes* which had high cytotoxicity on T47D and MCF7. It had highest cytotoxicity with the IC₅₀ on T47D cell was 19µg/ml and the IC₅₀ on MCF7 cell was 7 µg/ml done by (Yuyun Farida *et al.*, 2007). This findings suggests that combination methods were effective and efficient way to select marine actinomycetes producing potential compounds as anticancer.

CONCLUSION

Synthesis of silver nanoparticles using marine source has been unexplored, which aroused our interest in the present investigation. In the present study, it can be concluded that the identified species was *streptomyces grieseorubens*, one of the important source for producing effective silver nanoparticles by reducing silver nitrate and a good source for the extracellular synthesis of silver nanoparticles. These silver nanoparticles exhibited a tremendous potential anti-bacterial activity against common human pathogenic bacteria and has free-radical scavenging and anti-cancer activity on MCF-7 cell line.

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covers several different structural types of natural product molecules from biodiverse organisms with potential use as anticancer agents.

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