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Bioproduction and Characterization of Silver Nanoparticles from Marine Streptomyces sps KN11.

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ABSTRACT

Marine environment is a resource reservoir of biodiversity and the microorganisms occupy the special status in the marine biota because of their extreme physical and chemical adaptation. The most commonly occurring microbes in marine environment are marine bacteria, actinobacteria and fungi. Out of these marine microorganisms, actinobacteria are considered to be the potential producers of bioactive compounds. Majority of the actinomycetes are involved in the synthesis of the nanoparticles which is a recent trend in the development of novel pharmaceutical compounds. In the present study, Streptomyces sps. was used as a model for the bioproduction of silver nanoparticles. The silver nanoparticles were synthesized by the addition of 1mM $AgNO_3$ to the cell free supernatant of *Streptomyces Sps*. After 24h incubation, the silver nanoparticles were identified based on the colour and absorption maxima at 410nηm by using UV-Vis spectroscopy. The identified silver nanoparticles were characterized by using particle size analyzer (98.5nηm). TEM micrographs represented the presence of variable nanoparticles and their stability was analysed by zeta potential. The results provide the evidence that the extracellular factors from the cell free extract facilitates the synthesis of microbial nanoparticles.

Keywords: Silver nanoparticles, *Streptomyces*, Zeta potential, Antioxidant activity.

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INTRODUCTION

Nanoscience is gaining ever increasing popular attention due to its varied application in the newly emerging areas of research such as medical imaging, molecular diagnostics, electronics, space technology [1] and drug delivery system. Nanoscience extensively deals with the small structures, surface morphology and mono dispersity of nanosize particles. The nanoparticles, due to their surface to volume ratio, exhibit novel properties which are quite different from that of their macroscopic counterparts [2].

Nanoparticles can be synthesized either by top-down or bottom-up approach. Literature is abounding with the utilization of noble metals such as silver, gold and platinum as nanoparticles Among all metallic nanoparticles, the silver nanoparticles are of specific interest due to their therapeutic properties [3, 4]. The nanoparticles exert therapeutic applications due to their increased surface energy and catalytic reactivity. However, in most of the physical and chemical methods, hazardous chemicals and high energy requirements are applied for the production of nanoparticles [5]. Currently, green synthesis by exploiting natural materials like plants [6] bacteria [7,8] fungi [9,10] and yeast, has been proposed as an alternative to chemical and physical synthesis to develop low cost, non- toxic, high yield and eco-friendly nanoparticles.

Researchers started utilizing biomass or cell free extracts of actinobacteria for synthesis of nanoparticles due to their remarkable ability to reduce heavy metal ions. Actinobacteria are considered as nanofactories for the bioproduction of different compounds of nanometer size [11]. Actinobacteria are filamentous gram positive spore formers widely distributed both in terrestrial and marine ecosystem and considered as the most economically and pharmaceutically price less prokaryotes because of their capacity to produce secondary metabolites with potential applications.

The actinobacteria includes streptomycetes, actinomycetes, corynebacterium, frankia, micrococcus and others [12]. Among all, *Streptomyces* occupy the predominant position. The present study is designed for the biosynthesis and characterization of silver nanoparticles synthesized from marine *streptomyces sps* and also for analyzing the antioxidant and antimicrobial potential of these silver nanoparticles.

MATERIALS AND METHODS

Screening of actinobacteria for production of silver nanoparticles:

Marine sediment samples were collected from different location of Kotta Koduru of Nellore district (A.P) from depth of 5m in sterile bottles. The samples were air dried in laminar air chamber for 24hr and then dried in hot air oven for about 25mins at 70ºC.About 10g soil was serially diluted and inoculated on to nutrient glucose agar plates supplemented with 5mg/ml Rifampicin and 25mg/ml Nystatin to minimize the growth of bacteria and fungi. The screened isolates were identified up to the genus level based on their colony and biochemical characteristics. The identified isolates were inoculated into 100ml of Bennet broth(yeast extract 1g/L, beef extract1g/L, casein 0.5g/L (PH7.3) and incubated at 28^0c for 7 days under shaking conditions to produce biomass. The culture medium was harvested by centrifugation at 10,000 rpm for 10 min and the cell free extract was mixed with 1mM AgNO3 for the production of silver nanoparticles.

Characterization of microbial nanoparticles:

The bio reduction of silver nanoparticles by actinobacteria was detected with visual observation of color change from yellow to brown. Further, the production of silver nanoparticles was established by measuring the spectral changes in the wave length range from 300-700nm by using UV-Vis spectrophotometer (Shimadzu UV-1601) with Bennet broth as reference control.

Determination of Particle size of and stability of nanoparticles:

The particle size of the silver nanoparticles was analysed using particle size analyzer (Horiba SZ-100).Dynamic light scattering (DLS) is useful to measure the Z-average size of nanoparticles as hydrodynamic diameter estimated from the measured diffusion co-efficient by using the Stokes-Einstein equation .The stability of nanoparticles , reflected by zeta potential , was measured by using Zeta analyzer (Horibe SZ-100).

Fourier Transform Infrared Spectrometer (FT-IR):

FT-IR Analysis was carried out in order to determine the chemical groups involved in the formation and stabilization of silver nanoparticles. The measurements were carried out using a FT-IR- 3600 infra-red spectrometer.

Transmission electron microscopy:

The size and morphological features of the silver nanoparticles were analyzed using a Transmission electron microscope (Technai, F30). The samples were prepared by dispersing nanoparticles in 2% uranyl acetate solution for 3 to 4 h. About 4 to 6µl of the dispersed nanoparticles were dropped on a carbon coated copper grid (300 sqcm)and dried in air before exposure to the microscope.

Antimicrobial activity of silver nanoparticles:

The antimicrobial activity of silver nanoparticles was measured based on their activity on the growth of gram positive *such as Stapylococcus aureus*, *Bacillus cereus* and *E.coli, Pseudomonas aureoginosa as gram negitive* bacteria by Kirby-Bauer disc diffusion method on Mueller-Hinton agar with slight modifications[13].The synthesized silver nanoparticles were dispersed in water and nanoparticles in different concentrations ranging from 5 to 50µg/disc were transferred onto the sterile 6mm disc and incubated at 30ºc for 24 h. Control measurement was carried out with water. The zone of inhibition was determined by measuring the diameter of the clear zone(including disc) on the agar medium. All the experiments were carried out in triplicates and zone of inhibition was recorded.

DPPH free radical scavenging activity of silver nanoparticles

The antioxidant potential of silver nanoparticles was assessed by DPPH assay (2,2-Diphenyl-1-picrylhydrasyl) was determined [14]. An antioxidant reacts with DPPH by donating hydrogen and reduces DPPH. Briefly, 1ml of 0.2mm DPPH solution in methanol was mixed with different concentrations of (5-50µg/ml).The reaction mixture was vertexed and kept in dark for 15 minutes at 37^0C and the absorbance was measured at 517nm The percent of radical scavenging activity was calculated .

RESULT AND DISCUSSION

In our study, three actinobacteria designated as KN5, KN11, KN12 were enumerated from the marine sediment samples collected from sea coast of Nellore dt. AP India. The isolates were inoculated into Bennet broth for the bioproduction of silver nanoparticles. Among the three distinct morphological strains, only isolate KN11 showed the ability to synthesize silver nanoparticles and used for the further study. Based on the morphological and biochemical properties, the isolate KN11was identified as Streptomyces species (data not shown).

The cell free extract spiked with AgNO3 showed visual color change from colorless to dark brown after 24h of incubation with *Streptomyces sps* KN11. And color change was not observed in the absence of *Streptomyces* sps KN11.The colour intensity was increased with increase in time. Silver nanoparticles exhibit brown colour due to the excitation of surface plasm resonance [15].The silver nanoparticles produced from *Streptomyces sps* KN11 exhibited a characteristic absorption peak at 410nm (Fig 2) specifies the role of *Streptomyces sps* in the bio reduction and the production of nanoparticles. Our results are correlated with the extracellular synthesis of silver nanoparticles from other actinobacteria [16, 17].The bio generated nanoparticles showed a characteristic absorption peak at 410 nm. The data is in agreement with the marine isolate S. Albidoflavos CNP 10 with an absorption maxima at 410 nm.The location of absorption peak depends on the particle size and stability of the molecule [6].The size of the silver nanoparticles was measured by using particle size analyzer based on the scattering of dynamic light. As shown in(Fig.3) the particle size count was totally absent from 1nm to 60nm and it was initiated around 70nm gradually increased and finally the particle count reached to maximum at 100nm.These findings clearly indicates the average size of nanoparticles (95.8nm). The silver nanoparticles exhibited zeta potential of -19.5 mv. From the literature, it is noticed that the particles with zero potential values greater than +23mv and less than -20mv are considered to be stable [15].

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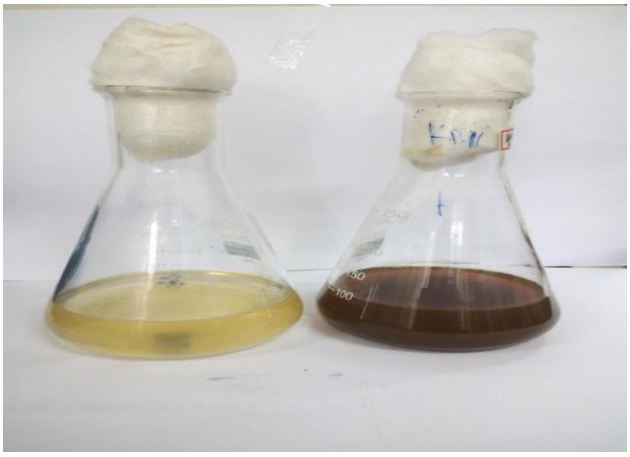

Figure 1: Synthesis of Silver nanooarticles (a) Bennet broth with AgNO3 (b) Bio reduction of silver ions by *streptomyces sps*.KN11

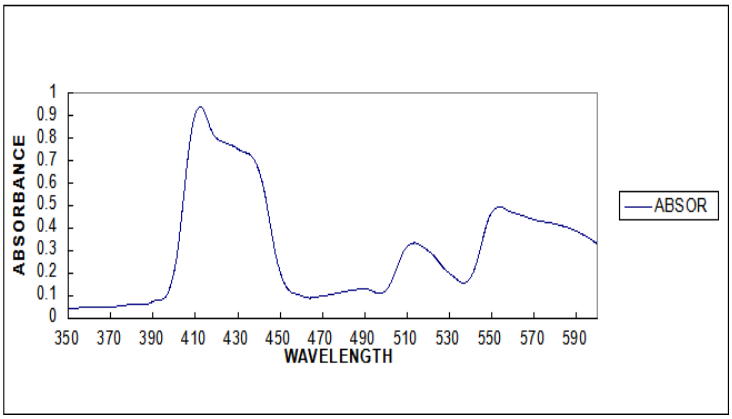

Figure 2: UV-Visible spectra of silver nanoparticles synthesized from *Streptomycessps*. KN11.

The stability and surface charge of colloidal silver nanoparticles was assessed by analyzing the magnitude of zeta potential. The microbial silver nanoparticles showed zeta potential of -19.5mv confirming the stability as well as non-aggregation of silver nanoparticles (Fig 4).TEM analysis was conducted to determine the surface morphology and size of the colloidal silver nanoparticles derived from *Streptomyces sps* KN11.The TEM micrographs exhibited the presence of spherical nanoparticles and the average particle size is in the range of 9 to 29.3ηm (Fig.5).

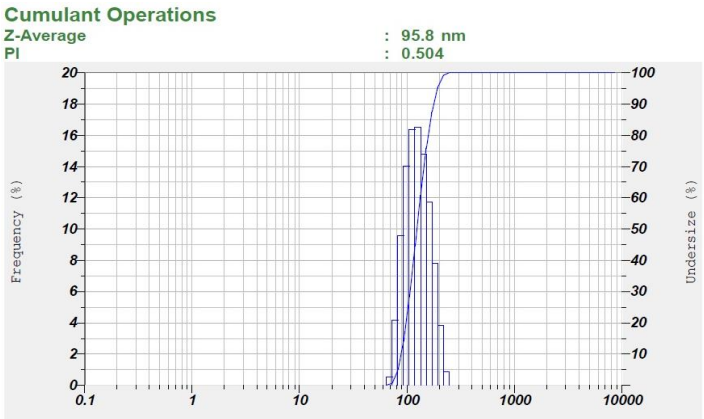

Figure 3: Particle size analysis of silvernanoparticles synthesized from *Streptomyces sps.KN11*.

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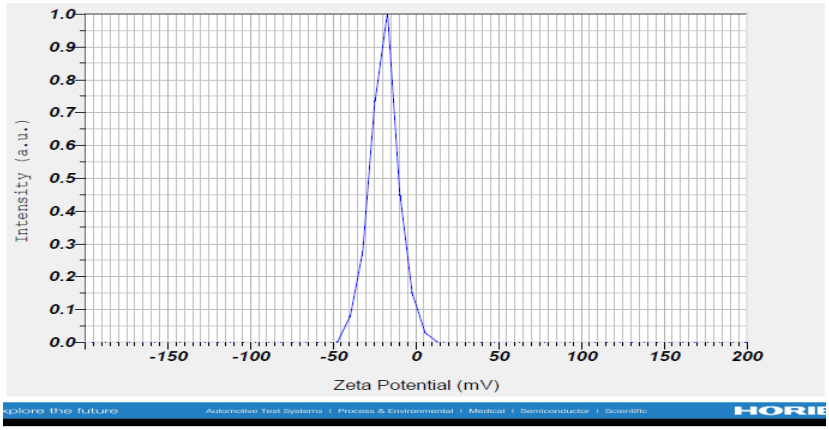

Figure 4: Zeta potential of *Streptomycessps* KN11derived silver nanoparticles

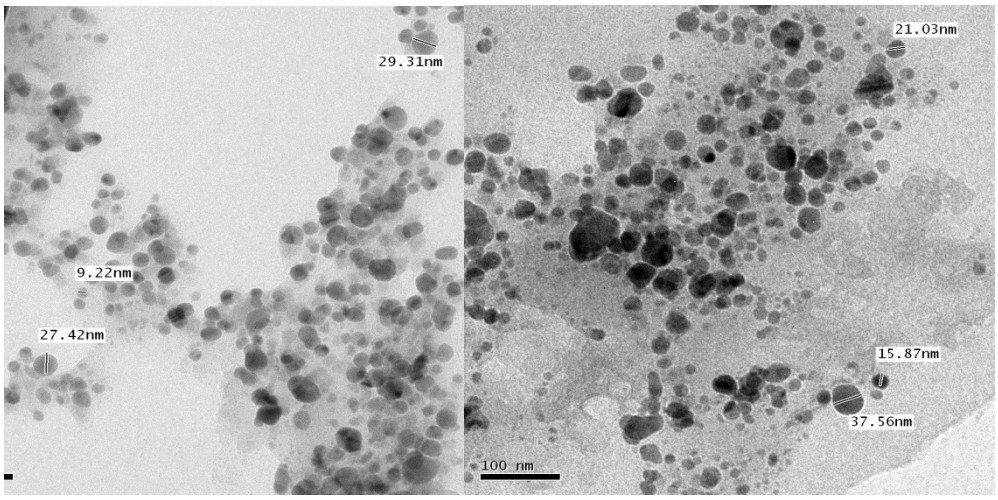

Figure 5: TEM micrographs of silver nanoparticles synthesized by *Streptomyces sps*. KN11

FTIR analysis was performed to identify the possible interaction between silver salts and the extracellular proteins of Streptomyces which is responsible for bio reduction of silver ions and capping of the silver nanoparticles. FTIR spectral analysis showed an array of absorbance bands in 500 cm^{-} to 3500 cm^{-1} (Fig 6).The peptide bond formation between different amino acid residues in a protein provides a characteristic spectral signature in the infrared region of the electromagnetic spectrum. The finger print at 3378 cm̄1 corresponding to the stretching vibrations of primary amines and 2926 cm̄¹ characterizes the hydroxyl group of carboxylic acid. The finger prints at1659 cm̄¹ and 1043 cm̄¹ indicates C=O group and C-N stretching vibration of the amine respectively. The occurrence of these signature peaks supports the presence of proteins in cell-free filtrate as observed in UV-VIS.spectra. The spectral data from FTIR strongly supports the bio reduction of silver salt by extracellular proteins mainly through the formation of peptide bonds. Our results correlating with earlier conclusions which indicates the presence of primary amines [18]

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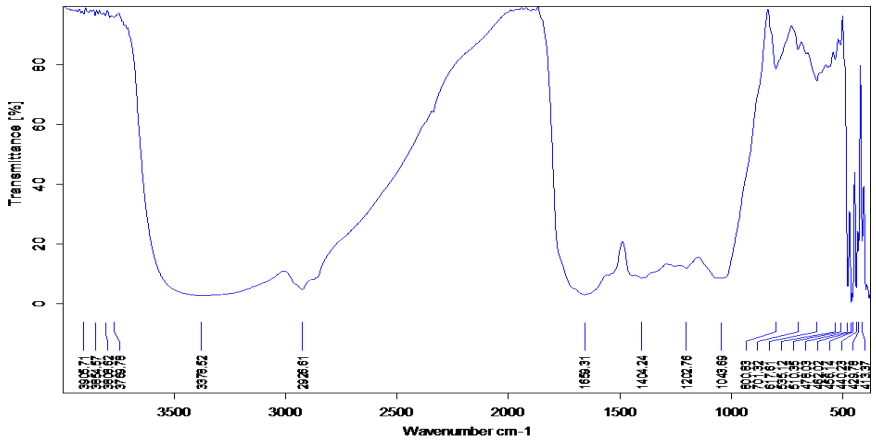

Figure 6: FTIR fingerprint of bio-reduced silver nanoparticles

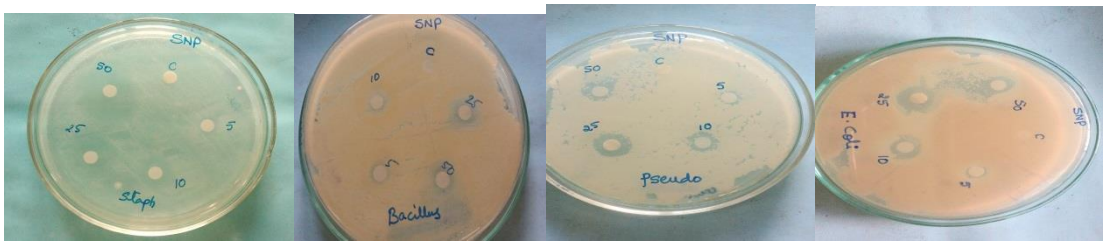

Figure 7: Antibacterial activity of silver nanoparticles derived from *streptomycessps*. KN11
(a) *Staphylococcus aureus* (b) *Bacillus cereus*
(c) *Pseudomonas aeruginosa* (d) *Escherichia coli*

The antimicrobial activity of silver nanoparticles at was tested against human pathogens by using streptomycin as a control. As per the data shown in Table-1, the silver nanoparticles exerted antibacterial activity against all pathogens and the minimum inhibitory concentration of silver nanoparticles was found to be 5µg/disc with Bacillus and 50µg/disc with Staphylococcus aureus and E.coli. The antimicrobial mechanism of biosynthesized silver nanoparticles may differ from species to species of bacteria [19, 20]. The synthesized silver nanoparticles were more effective against gram positive bacterial strain than the gram negative bacteria due to difference in the cell wall components [21, 22].Our results are in tune with published data to confirm the role of nanoparticles as bactericidal nanoparticles.

Table 1: CDOCKER energies and the number of hydrogen bonds between the ligands and NF-κB

Receptor	Ligand	No. of Hydrogen Bonds	Amino acids involved in Hydrogen bonding	Bond distance (Å)	CDOCKER interaction energy (kcal/mol)
NF-κB	cis-9, trans-11 CLA	2	LYS241	1.83	-51.75
			LYS272	1.94	
	trans-10, cis-12 CLA	1	LYS218	1.85	-54.85

The DPPH assay is a simple, fast and widely used method for measuring the free radical scavenging ability. Different concentrations of silver nanoparticles (25-100µl) were tested for the DPPH free radical scavenging activity. The percent scavenging of DPPH radical was measured at 517nm and the IC50 value was calculated by plotting the percent scavenging of DPPH at the steady state. The quenching of DPPH was found to be 50% and 29% at 100µg and 25µg of silver nanoparticles respectively. The IC50 for DPPH scavenging was found to be 50.20µg/ml of silver nanoparticles as per the linear regression. The data confirms proton donating and free-radical scavenging ability of silver nanoparticles.

CONCLUSIONS

The results indicate that the marine derived *Streptomyces sps* as a good source for the extracellular synthesis of silver nanoparticles and the characteristic properties of microbial nanoparticles were ascertained through visual observation, absorption maxima, Particle size, electrophoretic mobility. The microbial nanoparticles exhibited a potential antibacterial activity against common human pathogenic bacteria and free radical scavenging activity.

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