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Research Article

Synthesis of Bio-Silver Nanoparticles Using Desert Isolated *Streptomyces Intermedius* and Its Antimicrobial Activity

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ABSTRACT
In this study, a haloalkaliphilic actinobacterial strain was employed for the biosynthesis of nanosized silver particles. We used *Streptomyces intermedius* isolated from the saline desert of Kachchh as a bionanofactory for the production of bio-active silver nanoparticles in an eco-friendly and inexpensive manner. The bio (AgNPs) showed unique physicochemical and biochemical properties. The properties of the biosynthesized silver nanoparticles were studied using spectroscopic techniques, Nanoparticle tracking analysis and electron microscopy approaches. The UV-visible peak was found at 430 nm. Bio-AgNPs was found to be spherical shaped with an average particle size of 55nm and zeta potential value of -20.4mV. The obtained nanoparticles were crystalline and stable in nature. The antimicrobial efficacy was evaluated using the resazurin assay against both *Bacillus subtilis* (Gram-positive) and *Escherichia coli* (Gram-negative) bacteria. The antimicrobial assay of the silver nanoparticles showed a higher activity against *Bacillus subtilis* than *Escherichia coli*.

KEYWORDS: Actinomycete; bio-silver; nanoparticles; antibacterial; resazurin assay

INTRODUCTION

Nanotechnology is a new emerging multidisciplinary science that influences industrial, agricultural and medical sciences [1]. Biologically inspired production of nanoparticles is emerging as a new era in nanotechnology. Top-down approaches like physical and chemical methods may require chemicals harmful to the environment and they are also cost-effective. In contrast to that, bottom-up approach might offer clean, non-toxic and environment-friendly alternatives. Bottom-up approach includes oxidation/reduction of metal ions by different enzymes and biomolecules secreted by biological systems [2].

The diversity of biological systems like plants [3] bacteria, fungi [4] and seaweeds [5] have been reported for the synthesis of AgNPs. However, among the various microorganisms, actinomycetes are less explored for the synthesis of Nanoparticles [6] and very few reports are

available on the actinomycetes mediated synthesis of AgNPs. Biosynthesis and characterization of silver and gold nanoparticles have been reported from actinobacteria including *Streptomyces* sp. [7], *Nocardia* sp. [8], *Thermomonospora* sp. [9] and *Rhodococcus* sp. [10]. AgNPs are known for their excellent catalytic, electrical and optical properties [11]. Besides these, ionic silver has a broad spectrum of antimicrobial activity against bacteria [12]. It also inactivates the essential enzymes and proteins responsible for RNA and DNA replication, and disrupt transport processes in drug-resistant microorganisms [13,14].

The present study aimed at the production and characterization of biogenic metal silver nanoparticles by haloalkaliphilic actinobacterial strain isolated from the saline desert of Kachchh and evaluation of its antimicrobial activity.

MATERIALS AND METHODS

Isolation and identification

The soil samples were collected at 10cm below the surface in sterile plastic bags and stored in a refrigerator at 4°C. The isolation technique was followed as per methods described by [15]. The isolated strain was genotypically identified by 16S rRNA sequencing analysis and sequence data were compared with other National Center for Biotechnology Information (NCBI) GenBank database sequences using BLAST (basic local alignment search tool) at NCBI server (http://www.ncbi.nlm.nih.gov/genbank).

Synthesis of Bio-silver nanoparticles

The isolated culture was inoculated into MGYP (pH 7.0) and incubated in the orbital shaker (150 rpm) at room temperature for 7 days. After incubation, the biomass was filtered through Whatman filter paper No. 1, and the filtrate was allowed to react with 2mM AgNO3 solution for the biosynthesis of silver nanoparticles. The observation of the color change indicates the formation of Bio-AgNPs in the culture solution. The media without actinomycetes culture was maintained as a control.

Characterization of synthesized Bio-AgNPs

The Bio-AgNPs were characterized using UV-Vis, FTIR, XRD, SAED, AFM, SEM, HR-TEM and DLS with zeta potential. The UV-Vis spectroscopic analysis was performed using Lab India UV 3000+, India. The FTIR spectroscopic analysis was carried out using a Nicolet Avathar-320 FTIR spectrometer (Nicolet Instruments, Madison) at a scan range of 400–4000 cm^{-1} with a scanning speed of 2mm/S. The completely dried samples were treated with spectral grade KBr for pelleting in the ratio of 1:50 and were used for the FTIR analysis. Structural characterization was carried out by XRD with Cu Kα radiation using Rigaku D/max 2550 X-ray diffractometer. X-ray peaks were recorded at 2θ ranging from 20° to 70° at a scan rate of 0.05°/S. Morphology and crystal structure of the particles were examined by SEM using SEM-Zeiss Evo MA 15, UK at an accelerating voltage of 20kV and HR-TEM using FEI Tecnai FE12, the USA at an accelerating voltage of 200 kV. Size distribution and concentration of nanoparticles were measured using Nanoparticle Tracking Analysis (NTA) Version 2.3 Build 0034.

Resazurin assay for assessing antibacterial activity of Bio-AgNP

E. coli and *B. subtilis* were grown in Luria Bertani broth (Hi-Media, India) and incubated at 37°C under shaking (100 rpm). Cells were harvested and centrifuged at 8000 rpm for 10 min to obtain pellets. 1x phosphate buffer saline was used to wash the pellet and to remove traces of media. The optical density of suspension was measured at 600 nm and was compared with McFarland standard to enumerate cell number in the pellet. Approximately $1x10^5$ cells were inoculated per well with 200 µl of LB medium. Different concentrations (2.5, 5, 10, 20, 30, 40 ppm) of AgNP were added into the wells along with 10 µL of resazurin indicator solution. Resazurin dye (7-hydroxy-3H-phenoxazine-3-one 10-oxide) has been broadly used as an indicator of cell viability in several cytotoxicity assays. Reduction of resazurin to resorufin correlates with the number of live organisms [16]. The absorbance is recorded at 570 nm, and the IC50 value is determined [17].

RESULTS

Identification of Nanoparticle producing strain and biosynthesis

The 16s rRNA sequencing of the isolate yielded 913 base pairs, and NCBI BLAST search analysis revealed that the sequence was 98% similar with *Streptomyces intermedius*. The role of desert isolated strain *Streptomyces*

intermedius for an extracellular synthesis of silver Nanoparticles was investigated by mixing equal amount of 2mM AgNO3 and culture supernatant. The primary observation for the synthesis was done by color change from pale yellow to brown which occurred within 4 hrs indicated the formation of AgNPs in the reaction mixture.

Characterization of the Bio-AgNPs

The characteristic surface plasmon resonance of the reaction mixture obtained at 430 nm further confirmed the presence of silver nanoparticles in the reaction mixture. Control without supernatant showed no colour change. FTIR spectra of the Bio-AgNPs showed a characteristic band at 3328 cm^{-1}, which is assigned to the surface O-H group. The atmospheric CO_2 asymmetrical stretching vibration resulted in the characteristic band at 2111 cm^{-1}. It was observed that the band at 1635cm^{-1} correspond to a primary amine N-H band stretch vibrations of the proteins reacted during the nanoparticle formation. The band position at 531cm^{-1} represents the Ag–N and Ag–O stretching modes, respectively. Amorphous nature of AgNPs was determined by XRD analysis. The peaks (111), (200), (231), (222) and (311) in the spectra (Fig. 1a) reveal that the nanoparticles correspond to face-centered cubic (fcc) crystalline phase. This is further confirmed by the diffraction spots obtained in the SAED pattern which is shown in Fig. 1b.

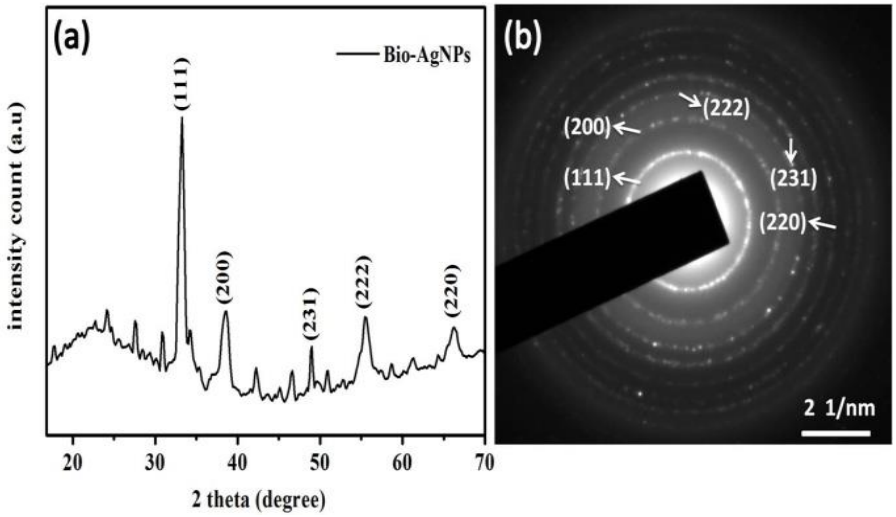

Fig 1: XRD and SAED pattern of Bio-AgNP

The SEM Fig. 2(a) and TEM Fig. 2(b) are used to study the morphology and size of nanomaterials. The SEM micrograph of the air-dried Bio-AgNPs shows the fairly dispersed spherical particles. The size distribution based on the hydrodynamic diameter and concentration of silver Nanoparticles in the reaction mixture was measured by Nanoparticle tracking analysis (NTA). NTA is based on light scattering and Brownian motion characteristics of particles. It measures the size of particles individually on a particle-by-particle basis. The average particle size was found to be 55 nm [Fig. 2(c)]. Zeta potential (f) is defined as the electrical potential between the inner Helmholtz layer near a particle's surface and the bulk liquid in which the particle is suspended. Zeta potential represents the charge of a particle and indicates the stability of the colloidal system. Large negative or large positive zeta potential is responsible for the repulsion of particles in the system thereby increasing the stability of colloidal solution [18]. The particles showed an average negative zeta potential value of 20.4 mV shown in Fig. 2(d).

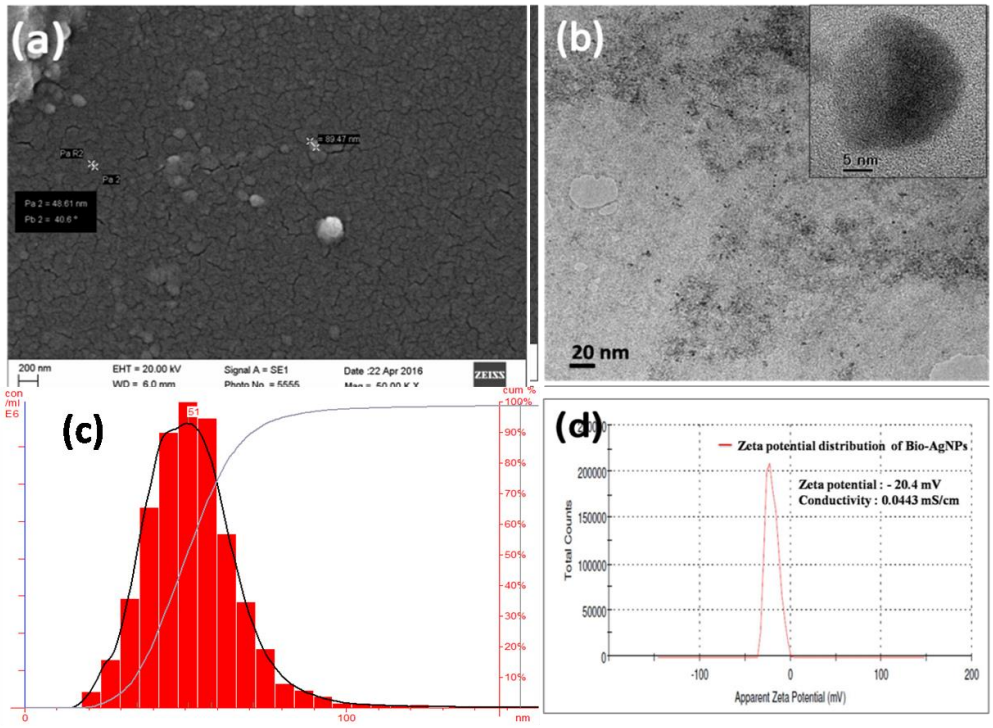

Fig 2: SEM (a), TEM (b and insert), DLS (c) and Zeta potential (d) of Bio-AgNPs

Antibacterial activity

Antibacterial assessment of silver nanoparticles was carried out using Resazurin assay for bacterial cell viability. It was observed that silver nanoparticles when treated with *E. coli* and *B. subtilis* inhibit cell growth. As presented on the graph (Fig. 3) with the increase in the concentration of Bio-AgNPs from 2.5 to 40 ppm there is a decrease in cell viability for both *E. coli* and *B. subtilis* which is a concentration-dependent phenomenon. It was observed that more inhibition was observed in the case of *B. subtilis* as compared to *E. coli*. The IC50 value of Bio-AgNPs was found to be 10 ppm for *B. subtilis* and 20 ppm for *E. coli*.

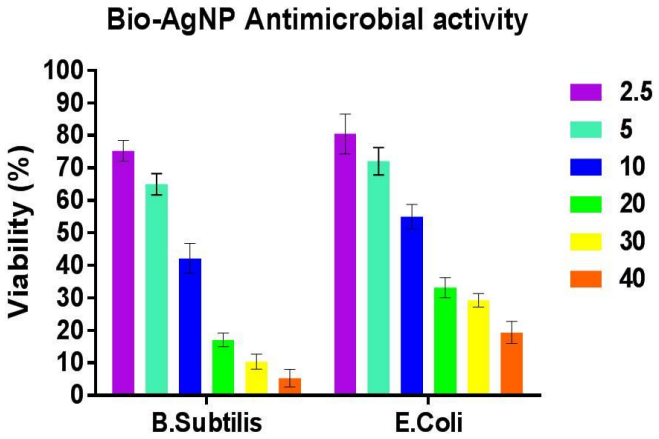

Fig 3: Resazurin assay for antibacterial activity of Bio-AgNP.

DISCUSSION

Synthesis of AgNPs from *Streptomyces intermedius* strain

Biological synthesis of nanoparticles does not require the use of hazardous chemicals and it is cost effective method [18]. Nanoparticles obtained by biogenic route are less toxic to human and environment [19]. Many authors have reported the extracellular biosynthesis of silver nanoparticles from different isolates [20,21]. Moreover, the role of intracellular components for the biosynthesis of nanoparticles has been reported by [22].

Characterization of biogenic silver nanoparticles

UV-visible spectroscopy is the most widely used technique for the structural characterization of nanoparticles. Silver Nanoparticles exhibit characteristic surface plasmon resonance in the range of 380-450 observed by [23,24]. AgNPs present in the reaction mixture showed surface Plasmon resonance peak at 430nm with a colour change from yellow to brown. The brown colour is formed by the excitation of surface plasmon resonance of silver nanoparticle [25]. A similar observation has been reported for *Bacillus licheniformis* [26], *Pseudomonas aeruginosa* [27], *Escherichia coli* [28], *Bacillus megaterium* [29]. Uv-visible peak also reveals the size, shape, and dispersity of nanoparticles in the reaction mixture which is based on Mie's theory [30]. The presence of a single peak indicates the presence of spherical particles whereas two or more peaks attributed to disc triangular shape [31]. The similar Uv-spectra for silver nanoparticles has been reported by [26,32].

FT-IR spectral data showed the presence of a variety of functional groups attached with the nanoparticles which may contribute to the synthesis and stabilization of nanoparticles. A similar conclusion was made by [33].

The size distribution of nanoparticles is a very important property for application purpose. For example, smaller the size of the particles greater the effective antiviral agents they are[34]. Potent antimicrobial activity of silver nanoparticles against *Streptococcus pneumoniae* was reported by [35] having a size of 12.3nm. The difference in size distribution properties of nanoparticles by different microbes may be the result of different reductase and coating proteins [22].

The comparison of the XRD spectrum of the silver nanoparticles with the spectra published by Joint Committee on Powder Diffraction Standards (File no 04-0783) confirmed the pure crystalline nature of the formed particles in the experiment. Similar XRD spectra have been reported by [36].

Antibacterial activity

Silver and silver ions are known to have strong inhibitory and antibacterial effects, as well as a broad spectrum of antimicrobial activities. Antibacterial activity of silver nanoparticles against pathogenic strains of *E. coli* and *S. aureus* has been reported by [37]. Additionally, anti-MRSA potential of silver nanoparticles formed by *Streptomyces exfoliates* has been reported by [21] in which they reported >2 ug/ml of MIC.

The general mechanisms behind the inhibition of the bacteria by the silver nanoparticles have been given in the Fig.7. Silver inhibits the respiration process by binding to the cell membrane and bacterial cell wall. Briefly, the Bio-AgNPs are known to mainly inhibit thiol group-containing enzymes, such as NADH dehydrogenase in the respiratory system, which is concerned as a candidate for the production of reactive oxygen species [38]. Therefore, inhibition of this particular enzyme results in an augments in the free radical production. It is proposed that reactive oxygen species can induce apoptotic pathways in bacteria which could ultimately direct to their death [34].

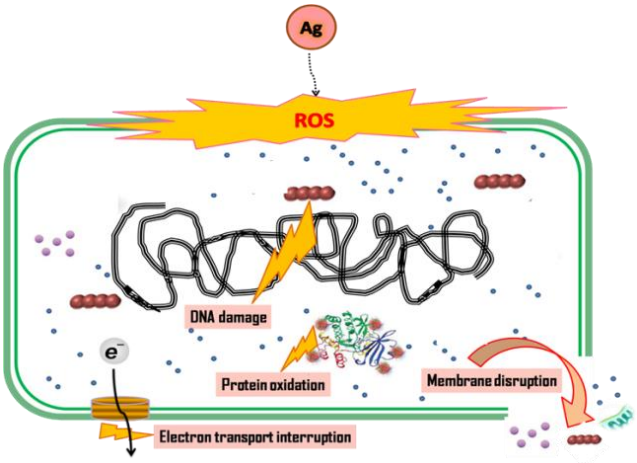

Fig 4: Proposed Mechanism of antibacterial activity by Bio-AgNPs

CONCLUSION

The microbial route applied for the biosynthesis is a reliable and eco-friendly approach. The actinobacterial strain employed in this experiment proved to be a good bio-reducing agent. On the basis of results, we can conclude that silver nanoparticles are powerful nano weapons in the medical sector.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest in this research article.

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