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ANTIBACTERIAL POTENTIAL OF BIOLOGICALLY REDUCED SILVER NANOPARTICLES FROM *STREPTOMYCES* sp. SO-01

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ABSTRACT

The syntheses of metallic nanoparticles were gaining importance because of their budding applications in the field of nanotechnology, microbial biotechnology, delivery of drug etc. To procure this, use of natural sources like biological systems becomes cost-effective, reliable and eco-friendly. In the current investigated, we have reported extracellular biological reduction of silver nanoparticles from 10^{-3} M silver nitrate using Streptomyces sp SO-01 isolated from Western Ghats. Biosynthesized silver nanoparticles were confirmed by UV-Visible spectroscopy and the spectra showed a maximum absorption at 430 to 440 nm corresponding to the Surface Plasmon Resonance. The silver nanoparticles were analyzed for their antibacterial potential on human pathogens Gram positive bacteria (Bacillus subtilis, Bacillus cereus, Streptococcus pyogenes, Staphylococcus epidermidis, Staphylococcus aureus) and gram-negative bacteria (Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi, Vibrio cholerae,). The maximum zones of inhibition of 15mm were observed against gram positive bacteria (Bacillus subtilis and Streptococcus pyogenes) and 18mm in gram negative (Vibrio cholerae and Salmonella typhi). This research gives a novel approach to developing new formulation based on metallic nanoparticles with antibacterial properties to reach the pharmaceutical companies searching for current uncustomary antibacterial agents.

KEY WORDS

Silver Nanoparticles, Streptomyces sp., UV-visible spectroscopy, Antibacterial potential.

INTRODUCTION

Nanoparticles serve as the radical building blocks for diverse nanotechnology applications [1]. Nanotechnology and alongside nanostructured materials play an increasing role in science, research and development, as well as also in day today's life, as more products based on nanostructure materials are introduced to the global market [2]. Nanotechnology assign with materials with dimensions of nanometres. Nanoparticles fall into two categories: organic and inorganic nanoparticles (like gold and silver) [3]. Silver nanoparticles reveal a rare combination of valuable properties including, unique optical properties associated with the surface Plasmon resonance (SPR), well-developed surfaces, catalytic activity, high electrical double layer capacitance etc. Since use glass windows with tiny colour metal particles of silver which furnish glassy yellow colours [4].

Silver is worn as a catalyst for the oxidation of methanol to formaldehyde and ethylene to ethylene oxide. Biologically reduction methods have been enhancing the performance of nanoparticles, properties with the aim to have a better control over particles size, distribution, morphology, purity, quantity and quality, by employing as environment friendly economical

processes has always been a challenge for the researchers [5].

Microbial origin to produce the silver nanoparticles shows the great appeal towards the precipitation of nanoparticles due to its metabolic activity [6, 7]. Specifically, compared with bacteria and fungi, the Actinomycetes are known to secrete proteins in giant amounts, there by significantly increasing the productivity of biosynthesis [8]. Actinomycetes are extensively distributed in soil and constitute a consequential part of soil microflora. Western Ghats are one of the thirty-four biodiversity hotspots in the world. They have been opulently known for their flora and fauna [9]. Soil Actinomycetes are prokaryotes with extremely various metabolic possibilities. Actinomycetes are gram positive filamentous bacteria, characterized by the formation of aerial mycelium and spores on solid media with DNA high in G+C content of 60-70 mol% [10]. The capacity of *Streptomycetes* to produce new compounds remains unravelled by the members of other microorganisms [11].

In an attempt to annotate the mechanism favouring for formation of nanoparticles with desired features, that exceptional biological condition such as alkaline and slightly elevated temperature conditions [12]. Silver nanoparticles interacting with the bacteria's, Silver ions retard the bacterial enzymes responsible for energy metabolism and electrolyte transport. Silver ions prevent bacteria proliferation by establishing a fortification system, slowing bacterial growth [13].

In this present research work, biologically reduced silver nanoparticles were investigated using the *Streptomyces* species. This work implies extracellular synthesis of silver nanoparticles and characterization of particles by UV- visible spectrophotometer. We employed silver nanoparticles for anti-bacterial potential against human pathogens.

MATERIALS AND METHODS

Soil sampling and Screening of Actinomycetes

The rhizosphere soil samples were collected in sterile Zip lock plastic cover from a depth of 15 cm from Western Ghats in Karnataka, India. The samples were air dried for 6 to 7 days and ground in a mortar using pestle [14]. Isolation were performed by serial dilution using dilution plate technique. The dilutions were carried out up to 10^{-6} dilutions. Aliquots (0.1 ml) of 10^{-2}, 10^{-3},10^{-4}, 10^{-5} and 10^{-6} were spread on the Starch casein nitrate agar and Actinomycetes isolation agar (Himedia, Mumbai) plates. To minimize the bacterial and fungal growth, Fluconazole and Greseofulvin were added. The plates were incubated at 30±2^{0}C for 7 to 21 days. The isolates were grown on starch casein nitrate agar medium at 30±2^{0}C and stored at 4^{0}C for short term storage [9].

Morphological Characterization

The classifications of actinomycetes were originally based largely upon the morphological observations. Preferably, the *Streptomyces* species in the first few editions of Bergey's Manual. The Actinomycete isolates were characterized primarily by their colony morphology, spore colour, aerial mass colour and substrate mycelium, pigmentation appearance on the medium. The isolates were identified to genus level based on their spore chain arrangement by covers slip technique [9].

Staining

All the isolates were subjected to grams and acid-fast staining procedures [9].

Screening and Extracellular Synthesis of Silver Nanoparticles

The isolated Actinomycetes were screened for the dominant silver nanoparticle production and further biosynthesis of silver nanoparticles were carried out by using starch casein broth containing (M-media), soluble starch-2%, K2HPO4-0.2%, KNO3-0.2%, NaCl-0.2%, Casein-0.03%, MgSo4.7H_2O-0.005%, CaCo3-0.02%, FeSo4.7$H_2$0-0.001% at 30^{0}C in shaking condition. After 4-5 days of incubation the mycelia (biomass) were separated from the culture broth by filtration and the mycelia were washed thrice with distilled water under sterile conditions. The biomass were taken again in the Erlenmeyer flask containing 100ml sterile distilled H_2O and incubated for 24 hours and again biomass were filtered through whatman filter paper No.1. The cell free filtrates thus obtained were resuspended in 100ml of 10^{-3} M AgNO3 solution for reduction [8].

Characterization of Silver Nanoparticles

The bioreduction of silver ions were monitored by visual observation of colour change from yellowish to reddish brown and further were confirmed by sharp peaks shown by the absorption spectrum of this solution and recorded by using UV-Vis spectrophotometer [15] and observed for wavelength scanning between 300-700 nm [16].

Antibacterial Activity of Silver Nanoparticles

Antibacterial activity of silver nanoparticles synthesized by isolates were investigated against pathogenic five Gram positive bacteria (*Bacillus subtilis, Bacillus cereus, Streptococcus pyogenes, Staphylococcus epidermidis, Staphylococcus aureus*) and five-gram negative bacteria (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi, Vibrio cholerae*) by agar well diffusion method. Wells of size 6 mm have been made on nutrient agar plates using gel puncture and each well were loaded with 10^{-3} M of synthesized AgNps, were incubated at 37^0C for 24 hours. The zones of inhibition around the wells were observed and measured [17, 18].

Biochemical Characterization and Enzyme hydrolysis

Standard biochemical tests were employed the Indole test, Methyl red test, Voges – Proskauer test, Citrate utilization test, Urease test, Catalase test and Enzyme hydrolysis by Casein, Gelatin and Starch hydrolysis and Degradation of Cellulose to determine the potent silver nanoparticle synthesizing strait [19].

RESULTS AND DISCUSSION

Sampling and Screening for Actinomycetes

Two rhizosphere soil samples were randomly collected in sterile Zip lock plastic cover from Western Ghats in Karnataka, India (Latitude 14^0 13^{I} 46.6^{II} N and longitude 74^0 49^{I} 57.2^{II} E) [14] and air-dried soil samples subject to serial dilution and 16 visible colonies were obtained on petriplates. 05 colonies were selected for further studies.

Morphological Characterization

The results showed a diverse morphological characteristic with varied spore colour, colony morphology, substrate and aerial mycelium colorations (Fig 1). The spore bearing hyphae and spore chains is determined by cover slip method, isolates shows the straight- rectus, retinaculum apertum-open loops, hooks and spirals (Fig 2). Based on the spore chain arrangements the isolates were assigned to the genus *Streptomyces* sp. (Table 1) [9, 20].

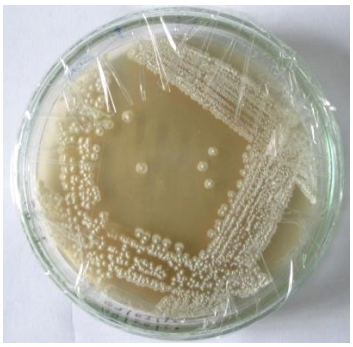


Figure 1: Pure culture plate of *Streptomyces* sp. SO-01

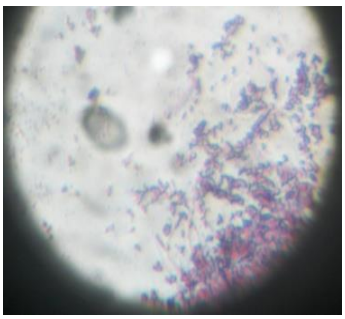


Figure 2: Microscopic view of *Streptomyces* sp. SO-01.

Table 1: Morphological Characterizations of isolates

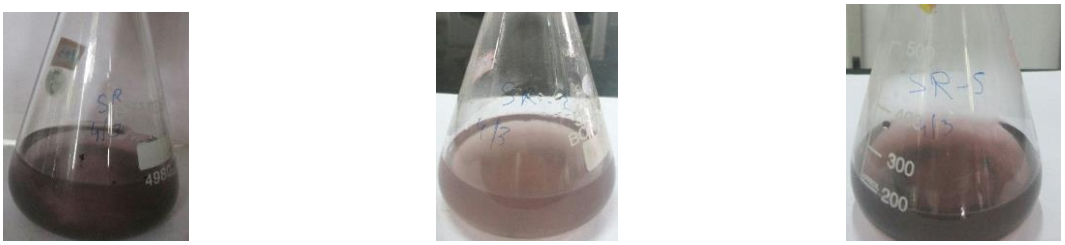
Isolates no.	Medium	Growth	Colony Morphology	Aerial Mycelium	Substrate Mycelium	Pigmentation	Spore Arrangement	Tentative genera
SO-01	SCN	Abundant	Powdery radiating	White	White	No	Ra-open loops	*Streptomyces* sp.
SO-02	SCN	Abundant	Velvety	Creamish	Grey	Pink	Ra-hook	*Streptomyces* sp.
SO-03	SCN	Abundant	Leathery	Cream	Brown	No	Straight-Rectus	*Streptomyces* sp.
SO-04	SCN	Abundant	Discrete	Dark Brown	Black	No	Sporangia	*Streptosporangium* sp.
SO-05	SCN	Abundant	Powdery	White	Grey	Brown	Ra-Extended	*Streptomyces* sp.

Staining

Selected five isolates were shown the gram positive, filamentous, rod structure, identified by gram staining and Nonacid fast [9].

Screening and Extracellular Synthesis of Silver Nanoparticles

The appearance of a yellowish to reddish brown colours (Fig 3) in the silver nitrate treated flask were clear indication of the formation of silver nanoparticles in the reaction mixture due to the reduction of metal ions and formation of surface plasmon resonance, whereas no colours change were observed in either the culture supernatant without silver nitrate [16]. Synthesis of AgNps after 24 hours by isolates SO-01, SO-02 and SO-05 showed good reduction of AgNps compared to SO-03 and SO-04 isolates (Table 2). Isolates were used for further characterization and application studies [8].



SO-01 **SO-02** **SO-05**

Figure 3: Biological Reduction Silver Nanoparticle filtrates by *Streptomyces* species.

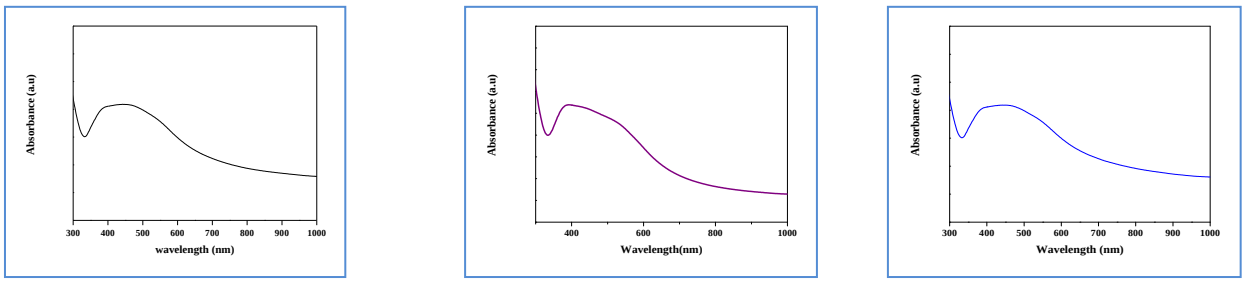
Table 2: Synthesis of Silver Nanoparticles

Isolates	AgN_{PS} Synthesized
SO-01	++++
SO-02	++
SO-03	+
SO-04	+
SO-05	+++

Characterization of Silver Nanoparticles

Silver Nanoparticles were successfully synthesized in the culture supernatant by isolates. The formation and stability of the reduced silver nanoparticles in the colloidal solution were monitored by a UV–vis spectrophotometer [21]. In the UV-visible spectrum (Fig 4) of the isolates SO-01, SO-02 and SO-5 filtrates show a strong peak were observed at 410 to 440 nm. The surface plasmon resonance (SPR) confirmed successful formation of AgNps [22].

It has been the bioreduction of silver ions occurred due to the presence of reducing agents such as electron shuttle quinines and enzyme reductase. Similar results were reported the Plasmon resonance of AgNps at 420 to 45 nm by *Streptomyces* sp. VDP-5 [8] and the absorption peak at 410 nm by *Streptomyces rochei* was observed by other researchers [23].



SO-01 **SO-02** **SO-05**

Figure 4: UV-Vis Spectra absorbance value of Silver Nanoparticles by *Streptomyces* species.

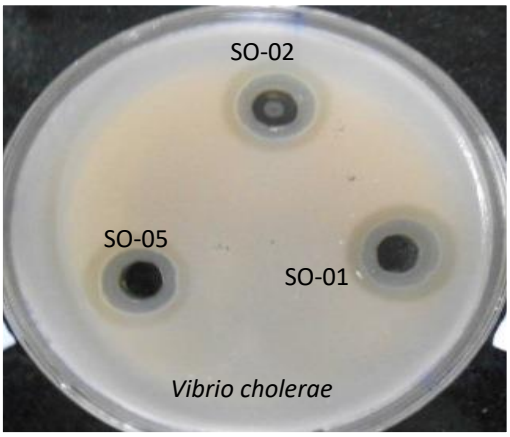


Figure 5

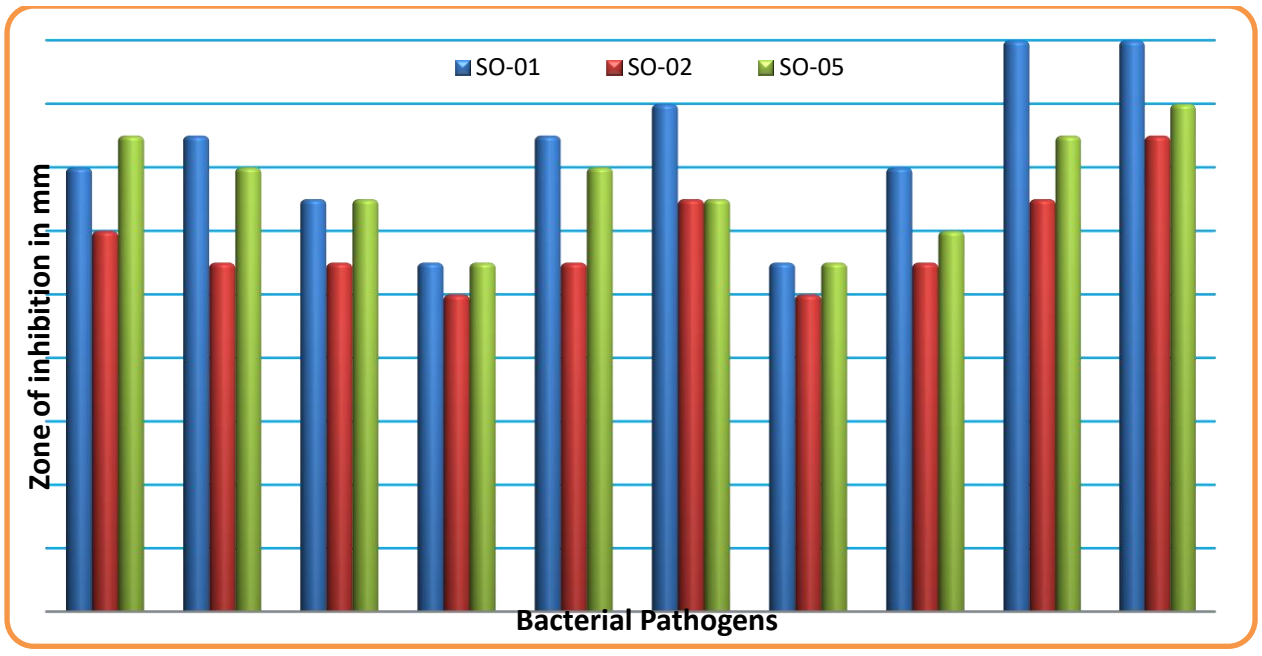


Figure 6: Antibacterial activity of silver Nanoparticles Synthesized by Streptomyces species.

Antibacterial Activity of Silver Nanoparticles

The silver nanoparticles obtained from isolates SO-01, SO-02 and SO-05 shows antagonistic activity against ten organisms of human pathogens to a large extent (Fig 5). The antimicrobial activity was reported to be due to the penetration of AgNps into the bacteria, damaging the cell membrane release of cell content.SO-01 shows the maximum zone of inhibition in mm compared to SO-02

and SO-05 for all the bacterial pathogens (Fig 6). Similar antibacterial activity of silver nanoparticles were investigated against some selected Gram negative (*Pseudomonas aeruginosa, E.coli, Klebsiella pneumonia, Enterobactor faecalis*) and Gram positive (*Staphylococcus aureus*) human pathogenic bacteria by agar well diffusion method [23].

Biochemical Characterization and Enzyme hydrolysis *Streptomyces sp.* SO-01

The biochemical characteristics as potent AgNps synthesizing shown (Table 4). The SO-01 exhibit positive methyl red, voges – proskauer, citrate, urease, catalase [19]. Enzyme hydrolysis of SO-01 also showed good hydrolysis upon Starch and Gelatin by producing amylase and protease respectively.

Table 4: Biochemical Characteristics and Enzyme hydrolysis.

Sl.no	Tests	*Streptomyces* sp. SO-01
Biochemical		
01	Indole	-
02	Methyl Red	+
03	Voges – Proskauer	+
04	Citrate	+
05	Urease	+
06	Catalase	+
Enzyme hydrolysis		
07	Casein	+++
08	Starch	++
09	Cellulose	-
10	Gelatin	+

Similar types of results were also observed enzyme hydrolysis of *Streptomyces* sp VDP-5 showed good hydrolysis upon Casein and starch [8]. Thus, proteins have the stronger ability to bind metal ions to stabilizing the nanoparticles.

CONCLUSION

Biological reduction of silver nanoparticles extracellurlly by using the *Streptomyces* species isolated from Western Ghats. The characterizations of silver nanoparticles were confirmed by UV-Visible Spectrophotometer. Silver nanoparticle shows a remarkable antibacterial potential against various pathogens. presently, good approach towards silver-based nanoparticles to be used in the field of medical for treatment of infectious diseases, Further investigation is needed to make simple, safely design, and eco-friendly silver nanoparticles alone or associated with antibiotic against diseases without harm for human and environment.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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