Characterization of Silver Nanoparticles Synthesized by Using Marine Isolate *Streptomyces albidoflavus*

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Silver nanoparticles production by the green chemistry approach was investigated using an isolated marine actinomycetes strain. The isolated strain was identified as *Streptomyces albidoflavus* based on chemotaxonomic and ribotyping properties. The strain revealed production of silver nanoparticles both extracellularly and intracellularly. Surface Plasmon Resonance analysis with the function of time revealed that particle synthesis by this strain is reaction time dependent. The produced particles were spherical shaped and monodispersive in nature and showed a single surface plasmon resonance peak at 410 nm. Size distribution histograms indicated production of 10–40-nm-size nanoparticles with a mean size of 14.5 nm. FT-IR spectra of nanoparticles showed N-H, C-H, and C-N stretching vibrations, denoting the presence of amino acid/peptide compounds on the surface of silver nanoparticles produced by *S. albidoflavus*. Synthesized nanoparticles revealed a mean negative zeta potential and electrophoretic mobility of −8.5 mV and −0.000066 cm²/V.s, respectively. The nanoparticles produced were proteinaceous compounds as capping agents with −8.5 mV zeta potential and revealed antimicrobial activity against both Gram-negative and -positive bacterial strains. Owing to their small size, these particles have greater impact on industrial application spectra.

Keywords: *Streptomyces albidoflavus*, silver nanoparticle, transmission electron microscopy, antimicrobial activity

Nanotechnology is an emerging cutting-edge interdisciplinary research. The application of nanoparticles in various industrial sectors is continuously increasing owing to particle size mediated acquisition of unique physicochemical characteristics, like a high surface area-to-mass ratio and high reactivity compared with its counterpart bulk material [14]. Various nanoparticles have been successfully employed in catalysis, pharmaceutical nanoengineering, drug delivery, sensor development, electronics, and allied sectors [5, 10, 17, 36, 38]. The application potential of these particles is mainly determined by their uniform size, shape, and crystalline nature in addition to their monodispersibility and stability [22, 24]. Although an array of physical and chemical methodologies are extensively developed, the synthesis of monodispersed nanoparticles with different sizes and shapes has been a challenge in nanotechnology. In addition, use of toxic substrates as desizing agents during chemical synthesis restricts their use in clinical and pharma applications, which is another subject of paramount concern [22]. Hence, the development of biocompatible, non-toxic, and environment-friendly nanoparticle synthesis technologies would be the desire of the scientific community.

Biological synthesis of nanoparticles is a green chemistry approach. Microbial properties of bioaccumulation, biosorption, biodegradation, and biomaterialization have been regarded as opportunity to use them as nanofactories [7, 19, 22, 26]. In this context, several microbial strains or plant cell extracts have been exploited as a simple and viable alternative to chemical and physical synthesis approaches. It was well documented that silver nanoparticle production could be possible using the cell mass of certain bacteria, fungi, and yeast strains, either extracellularly or intracellularly. However, microbe-specific variation in nanoparticle properties has been observed [29, 35]. For example, the time required for completion of nanoparticle production varies from 24 to 120 h. In addition, the size, stability, and dispersion properties of produced nanoparticles varied with the type of microbial strain employed. The production of pyramidal
and 5–200-nm-size silver nanoparticles by *Phaenerochaete chrysosporium* was reported [35], whereas *Coriolus versicolor* [29] produced spherical and 25–75-nm-size particles, and *Penicillium brevicaespactum* synthesized spherical shaped particles of 58.35 ± 18 nm size, indicating that the biochemical and genetic nature of microbial strain employed plays a significant role in controlling the nanoparticle biogenic processes [9]. Hence, scientific researchers worldwide are exploring microbial strains from xenobiotic environments to study the biosynthesis process of nanoparticles for industrial exploitation. Keeping this in view, the effort has been made to isolate a marine microbial strain having the potential for production of biocompatible, spherical, and highly monodisperse nanoparticles. The data revealed that the isolated microbial strain belongs to actinomycetes genera and produces an average mean size of 14.5 nm silver nanoparticles that have a polydispersity index of 0.798 with a zeta potential of −8.5 mV. The biosynthesized nanoparticles were characterized for their surface properties using FTIR and TEM in addition to antimicrobial property using Gram-negative and Gram-positive bacterial strains.

### MATERIALS AND METHODS

#### Isolation and Screening of Microorganism

Marine soil sediment samples were collected from the coast of Bay of Bengal near Visakhapatnam, Andhra Pradesh. The soil sediment was brought aseptically to the laboratory and enriched with starch-casein (1% w/v) broth under sterile condition by supplementing 0.25 g/l (w/v) yeast extract and 10.0 g/l (w/v) malt extract. The culture was incubated at 27°C for 72 h in a shaking flask at 225 rpm. The cell biomass was harvested by centrifugation at 10,000 rpm for 10 min for the identification of strain based on chemotaxonomic properties of mainly cell wall amino acid and carbohydrate content according to Cummins and Harris [6]. For cell wall amino acid composition, the biomass was treated with 6 N HCl at 95°C for 2 h and the digestate was analyzed for amino acid composition by paper chromatography using n-butanol, acetic acid, and water in the ratio of 60:25:15 as solvent. For cell wall carbohydrate analysis, the cell mass was treated for 2 h with 2 N HCl and the resultant digestate was analyzed by paper chromatography using propanol and water in the ratio of 9:1. Diaminopimelilic acid and glycine were used as referral compounds for amino acid analysis, and mannose, galactose, and ribose for carbohydrate analysis. Molecular-based characterization on rRNA sequence of 16S rRNA was performed at the Microbial Type Culture Collection Centre, IMTECH, Chandigarh, India.

#### Phylogenetic Analysis

Nucleotide sequences were compared with those maintained in the GenBank Database through NCBI Blast (http://www.ncbi.nlm.nih.gov). Alignment of nucleotide sequences was done using a cluster method of the DNASTAR software program (DNASTAR Inc., Madison, WI, USA). Data analysis was performed using bootstrapped dataset characterized with 1,000 replicates. In order to determine the genetic relationship between these strains, a phylogenetic tree was generated based on the percentage difference between the sequences using the neighbor-joining method in the MEGA 5.05 software.

#### Synthesis of Silver Nanoparticles

For silver nanoparticle biosynthesis, the selected microbial strain was grown in medium consisting of 7 g/l KHPO₄, 2 g/l K₂HPO₄, 1 g/l MgSO₄·7H₂O, 1 g/l (NH₄)₂SO₄, 0.6 g/l yeast extract and 10 g/l dextrose, adjusted to pH 7. The 72 h grown isolated marine microbial biomass was resuspended in sterile medium supplemented with AgNO₃ (1 mM) and without AgNO₃ in separate sets of experiments. The reaction mixture was further incubated at room temperature for 72 h under static and at 200 rpm and the silver nanoparticle production monitored.

#### Isolation and Characterization of Silver Nanoparticles

The biogeneration of the silver nanoparticles in the reaction mixture was measured by withdrawing 2 ml of the sample at predetermined time intervals and the absorbance was measured in the range of 360 to 440 nm at a resolution of 1 nm using a UV-Vis spectrophotometer (Beckman DU-40) against sterile medium as the blank. The produced silver nanoparticles were isolated from the reaction mixture by filtering through 0.2 micron size Whatman filter paper and the filtrate was subjected to centrifugation at 10,000 rpm for 60 min using a Kubota 3500 (Japan). The settled nanoparticles were collected and dried overnight.

#### Zeta Potential Analysis

Zeta potential analysis was carried out at scattered angle of 173° by laser diffractometry, using a HORIBA SZ100 zeta potential analyzer based on the principle of Laser Doppler electrophoresis. Measurements were obtained in the range of −200 to +200 mV.

#### Transmission Electron Microscopy (TEM) Analysis

TEM analysis of synthesized silver nanoparticles was prepared by drop-coating biosynthesized nanoparticles solution on carbon-coated copper TEM grids (400 µm × 40 µm mesh size). Samples were dried and kept under vacuum in desiccators before loading on to a specimen holder. TEM measurements were performed on a Tecnai-12 (FEI, The Netherlands) electron microscope operated at an accelerating voltage of 120 kV.

#### Fourier-Transform Infrared (FT-IR) Chemical Analysis

Fourier-Transform Infra-Red spectroscopy measurements, the biotransformed products present in extracellular filtrate were freeze dried and diluted with potassium bromide in the ratio of 1:100. The FT-IR spectrum of samples was recorded on a FT-IR instrument.
**RESULTS AND DISCUSSION**

**Preliminary Evaluation of Silver Nanoparticle Production by Isolated Actinomycetes Strains**

A total of 10 microbial strains belonging to Actinomycetes group, isolated from the soil sediments collected from the sea coast of Bay of Bengal near Visakhapatnam, were evaluated for production of silver nanoparticles using selective starch-casein medium supplemented with 0.25 and 0.05 μg/ml final concentrations of rifamycin as antibacterial and cyclohexamide as antifungal agent, respectively. The data revealed that all isolated strains grew well under submerged fermentation conditions using mineral salt medium. To select the best strain among the isolated strains, silver nanoparticle production was carried out using cell-free extract of 72 h grown fermentation broth with the supplementation of AgNO₃. Preliminary reduction of silver ions was identified based on the change of color of the solution/reaction mixture from pale yellow to brown and its constant increase to a brown color. However, little change in color was noticed with medium without biomass (negative control) and unfermented medium (positive control). The plates were incubated at 37°C for 24 h and then were examined for the presence of zones of inhibition. The diameter of such zones of inhibition was measured and the mean value for each organism was recorded and expressed in millimeter unit.

**Identification of Silver-Nanoparticle-Producing Actinomycetes Strain and Phylogenetic Tree**

Chemotaxonomic analysis of a selected marine actinomycetes isolate, CNP10, revealed the presence of two amino acids (i.e., diaminopimelic acid and glycine) indicating the selected microbial strain belongs to Type I Class of Streptomyces genus. To confirm further, the cell wall carbohydrates were analyzed. The results revealed no characteristic sugar (i.e., arabinose, mannose, and galactose) were present. Further identification of this strain was performed by ribotyping of 16S rRNA and the data revealed that this strain corresponds to *Streptomyces albidoflavus*.

The 16S rRNA gene sequencing analysis of the isolate yielded 1,263 base pairs, and NCBI BLAST search analysis based on the topology of phylogenetic analysis revealed that the sequence was 98% related with several *Streptomyces* sp. (Fig. 1). The developed tree based on the neighbor-joining method for obtained 16S rRNA sequences depicted that the isolate occupies a distinct phylogenetic position within the radiation including representatives of the *Streptomyces* family.

**Production of Silver Nanoparticles**

The imperative role of the isolated marine *S. albidoflavus* biomass and its extracellular fermentation broth on biosynthesis of silver nanoparticles was investigated separately by...
supplementing the 1 mM (w/v) silver nitrate in the cell-free fermentation broth and in the sterile medium consisting of produced stationary-phase biomass. The biosynthesis of silver nanoparticles was measured as a function of time by monitoring the surface plasmon resonance in the range 360 to 440 nm using a UV-Vis spectrophotometer. Time-dependent analysis of surface plasmon resonance indicated an increase in peak value with a function of time mainly at 410 nm in both conditions, and little change in positive (silver nitrate in fresh medium) and negative (medium containing biomass without silver nitrate) controls. These data further supported that the marine isolate, *S. albidoflavus* CNP10, has potential to reduce the silver ions to nanoparticles, and biosynthesis of metal nanoparticles is associated with the metabolic nature of the microbial strain employed. Khosravi-Darani [13] also observed similar UV spectra for solutions containing reduced silver ions with high absorption intensity. The data are in agreement with the results of silver nanoparticle production by stationary-phase *Bacillus licheniformis* [11], whereas Natarajan et al. [23] reported production of silver nanoparticles by mid-log phase cultures of *Escherichia coli*.

Characterization of Silver Nanoparticles

UV-Visible spectral analysis. To understand the produced nanoparticle physical properties, surface plasmon resonance spectra recorded in the range of 360 to 440 nm further suggested the presence of a single peak in both reaction environments (i.e., extracellular and intracellular production conditions) (Fig. 2). This suggested that the produced silver nanoparticles are spherical in shape. This is based on the fact that according to Mie’s theory [18], colloidal particle shape determines the number of surface plasmon resonance peaks and a single peak corresponds to spherical particles, whereas two or more peaks in this range are attributed to disc or triangular shape, respectively. Sosa et al. [33] also reported increase of the number of surface plasmon resonance peaks as the symmetry of the nanoparticles decreases. Such single surface plasmon resonance in the recorded spectral range suggested that the produced silver nanoparticles are in spherical shape, characterized with a monodispersive character.

Brause et al. [4], working with silver colloids in aqueous solution, reported that optical absorption spectra of metal nanoparticles are mainly dominated by surface plasmon resonance, and the absorption peak has relationship with particle size. The authors also concluded that the surface plasmon resonance peak of silver nanoparticles in aqueous solution shifts to longer wavelengths with increase in particle size. In context of the above, further analysis of surface plasmon resonance spectra of silver nanoparticles produced by the marine isolate revealed an absorption peak at 410 nm (Fig. 2), which is at a lower wavelength compared with literature reports. Natarajan et al. [23] reported a surface plasmon resonance peak at 410 nm for silver nanoparticles produced by bacterial strain *E. coli*, whereas a maximum peak at 420 nm for silver nanoparticles was noticed by Pal et al. [25].

Fig. 2. Surface plasmon resonance spectrum (UV-Visible) for silver nanoparticles produced by *Streptomyces albidoflavus*.
Transmission Electron Microscopy (TEM) analysis.
In Fig. 3A, the TEM picture represents the silver nanoparticles produced by *Streptomyces albidoflavus* by depositing the film on a carbon-coated copper TEM grid. This TEM image provided further insight into the morphology and size of the produced nanoparticles. It is evident from the figure that the biosynthesized silver nanoparticles are spherical in shape and mostly observed as individual particles as well as a few as aggregates. Such TEM mediated characterization of biosynthesized nanomaterials has been performed by several investigators [3, 12, 31]. However, nanoparticles were not in direct contact even within the aggregates, indicating stabilization of these particles by a capping agent, which are monodispersive in nature in aqueous environment. This corroborates the study of Ahmad et al. [2] using *Fusarium oxysporum* and Saifuddin et al. [28] using *Bacillus subtilis*. Particle size analysis revealed that the produced silver nanoparticles are in the size range of 10–40 nm with a mean diameter of 14.5 nm, suggesting production of different-size nanoparticles. Further analysis of particle size distribution revealed that approximately 28% of particles are 20 nm sizes (Fig. 3B). Productions of 35–46 nm silver nanoparticles by *Pseudomonas stutzeri* [15], 20–50 nm particles by *Lactobacillus* sp. [21], 2–20 nm particles by *Verticillium* sp., and 2–50 nm size particles by *Fusarium oxysporum* [2] have been reported. Further analysis of particle size distribution denoted that more than 75% of particles are in the range of 10–30 nm, suggesting this microbial strain has potential to produce low-size particles with high surface area. Analysis of polydisperse index revealed 0.798, indicating their distribution pattern in aqueous solutions.

Fourier-Transform Infrared (FT-IR) chemical analysis.
FT-IR spectroscopy was used to characterize the surface chemistry of silver nanoparticles produced by marine isolate *S. albidoflavus*. Fig. 4 shows the FT-IR spectrum of the freeze-dried powder of silver nanoparticles formed after 24 h of reaction. The spectral data recorded revealed two types of vibrations (i.e., stretching and bending) in the wavelength range of 4,000 to 500 Cm\(^{-1}\). It could be evident from the figure the presence of an amine vibration band at 3,296 Cm\(^{-1}\) representing a primary amine (N-H) stretching, and amide (N-H) bending vibration bands at 1,647 and 1,535 Cm\(^{-1}\). Furthermore, the FT-IR spectra of biosynthesized silver nanoparticles also revealed peaks at 2,965 and 2,931 Cm\(^{-1}\) stretching vibrations of aliphatic C-H bonds. A single band presence at 1,452 Cm\(^{-1}\) can be assigned to CH\(_2\) scissoring stretching vibration at the planar region. Several C-N stretching vibration peaks at 1,230, 1,183, 1,132, 1,098, 1,058, and 980 Cm\(^{-1}\) were also observed in the spectral range of 1,230 to 900 Cm\(^{-1}\). In addition, the presence of bands at 1,380 and 1,280 Cm\(^{-1}\) in the FT-IR spectra suggested the capping agent of biosynthesized nanoparticles possesses an aromatic amine groups with specific signatures of amide linkages between amino acid residues in the proteins in the infrared region of the electromagnetic spectrum, as reported by Shaligram et al. [31]. This type of FT-IR spectra supports the presence of a protein type of compound on the surface of biosynthesized nanoparticles, confirming that metabolically produced proteins acted as capping agents during production and prevented the reduced silver particles agglomeration. In fact, carbonyl groups from the amino acid residues as well as peptides are known for strong silver binding property. These data further indicate that the isolated marine, *S. albidoflavus* CNP10, produces extracellular protein compound that can bind to synthesized nanoparticles through free amine groups, as well as cysteine residues present in the protein, and thereby acting as a capping agent during synthesis of silver nanoparticles. Similar observation was reported by Balaji et al. [3] based on FT-IR spectra recorded for *Cladosporium cladosporioides* biosynthesized nanoparticles.

**Zeta Potential**
The biosynthesized silver nanoparticles were further characterized for zeta potential values by applying voltage...
across a pair of electrodes at either end of a cell containing the particle dispersion. Charged particles are attracted to the oppositely charged electrode and their velocity is measured and expressed in unit field strength as their mobility. Nanoparticles synthesized by the isolated marine *Streptomyces albidoflavus* revealed a mean negative zeta potential of \(-8.5\) mV, indicating \(-0.000066\) cm\(^2\)Vs\(^{-1}\) mean electrophoritic mobility (Fig. 5). Sadowski et al. [27], working with silver nanoparticles production by fungal strain *Penicillium* sp., reported that the produced particles zeta potential values were in function of pH. Further research activities are in progress to understand the impact of microbial metabolism and extracellular medium pH on production of different zeta-potential-containing silver nanoparticles.

**Antibacterial Activity**

Silver as an antimicrobial agent has been well documented since Roman time; however, this bactericidal property has been revived with the advancements in nanoparticle development. Although, the exact mechanism is not yet elucidated, its antimicrobial property has great potential against microorganisms, as the nanosilver along with their ambulatory surfaces would provide an alternative means to decrease the microorganism colonization and device-associated infection, including ventilator-associated pneumonia, central venous catheter infections, and catheter-associated urinary tract infections.

The antimicrobial activity of isolated *S. albidoflavus*-synthesized nanoparticles was assessed using two Gram-positive (*B. subtilis* and *M. luteus*) and two Gram-negative (*E. coli* and *K. pneumoniae*) bacterial strains by the well-diffusion method (Fig. 6A–6D). A control (cell-free fermentation broth without AgNO\(_3\) addition) was also maintained in each plate along with 30 µl of standard antibiotic streptomycin (30 µg/ml) in a separate well as positive control. The diameter of inhibition zones around each well with AgNPs is represented in Table 1. It was noticed that the produced nanoparticles revealed antibacterial activity against selected Gram-positive and Gram-negative bacteria (Table 1). The highest antimicrobial activity was observed against *K. pneumoniae* (36 mm), followed by *M. luteus* (28 mm) and *B. subtilis* (22 mm), and the least was noticed against *E. coli* (24 mm). The negative control (culture broth without AgNO\(_3\)) did not show any activity (Fig. 6), which further confirms the antibacterial activity of silver nanoparticles. Further comparative evaluation of the antimicrobial property of silver nanoparticles to that of standard streptomycin suggested that the antibacterial property of produced silver particles is almost comparable with streptomycin (Fig. 6). In fact, *Micrococcus luteus* was more susceptible to silver nanoparticles than to streptomycin. These data further supported that the antibacterial mechanism associated with silver nanoparticles may be different to that of streptomycin. These results are in agreement with literature reports on antimicrobial property of *E. coli* and *Streptomyces* sp. produced silver nanoparticles [8, 32]. Yamanaka et al., [37] reported silver nanoparticles mediated antibacterial activity against *S. aureus* and *E. coli*. The observed variation in zone of inhibition against *E. coli* and *K. pneumoniae* by biologically produced silver nanoparticles by Shirley et al. [32] and in the present study may be attributed to use of more than 50% less particles in the present investigation. In conclusion, this study revealed that the novel marine actinomycete *S. albidoflavus* CNP10 synthesized silver nanoparticles. The produced silver nanoparticles are spherical in shape with a monodispersive nature and showed single surface plasmon resonance at 410 nm. TEM studies revealed that the produced silver nanoparticles showed a size variation ranging from 10 to 40 nm with a mean size of 14.5 nm. The synthesized silver nanoparticles revealed

![Antibacterial activity of silver nanoparticles produced by *Streptomyces albidoflavus*.](image)

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Zone of inhibition (mm)</th>
<th>Silver nanoparticles(^a)</th>
<th>Streptomycin(^b)</th>
</tr>
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<tbody>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>24</td>
<td>24</td>
<td>28</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>22</td>
<td>22</td>
<td>25</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>36</td>
<td>36</td>
<td>40</td>
</tr>
<tr>
<td><em>Micrococcus luteus</em></td>
<td>28</td>
<td>28</td>
<td>26</td>
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\(^a\)Zone of growth inhibition diameter with silver nanoparticles.
\(^b\)Zone of growth inhibition diameter of streptomycin (positive control).
stretching vibrations of primary and secondary amines along with C-H and C-N. The metabolically produced protein compounds are involved in size regulation of reduced silver nanoparticles. An average negative zeta potential value of 8.5 was observed when characterizing the electrophoretic mobility. The biosynthesized nanoparticles revealed antimicrobial property against Gram-negative as well as Gram-positive bacterial strains. The isolated marine *S. albidoflavus* CNP10 with potential to produce silver nanoparticles can be exploited for bulk production of reproducible, monodispersive, spherical, and 14.5 nm mean size silver nanoparticles using a green approach for commercial application.

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