Biosynthesis of silver nanoparticles using marine Actinobacteria streptomyces albogriseolus and its antibacterial activity

ARTICLE *in* BIOTECHNOLOGY AND APPLIED BIOCHEMISTRY · NOVEMBER 2012

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Biosynthesis of silver nanoparticles using actinobacterium *Streptomyces albogriseolus* and its antibacterial activity

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Abstract.

An eco-friendly approach to the synthesis of silver nanoparticles (AgNPs) by extracellular components of *Streptomyces albogriseolus* has been reported. The isolated actinobacteria were genotypically identified by 16S rRNA sequencing analysis, and the morphology was observed by high-resolution scanning electron microscopy. The preliminary characterization of synthesized nanoparticles was carried out using ultraviolet–visible spectrophotometer. The maximum absorption spectra were found to be 409 nm at the 48th hour of incubation. The yield of AgNPs was found to be 72.64% as quantified by an atomic absorption spectrophotometer. The average size of AgNPs determined by the dynamic light scattering technique was 16.25 ± 1.6 nm. The results from transmission electron microscopy and X-ray diffraction confirmed the formation of spherical shaped and crystalline AgNPs. The interaction of protein with AgNPs was confirmed by Fourier transform infrared spectroscopy analysis. The biosynthesized AgNPs inhibited the growth of food pathogens (*Bacillus cereus*, *Escherichia coli*, and *Staphylococcus aureus*). Hence, the synthesis of AgNPs by *S. albogriseolus* could be employed as a probable antimicrobial agent to eliminate pathogenic microorganisms. This approach employed for the synthesis of nanoparticles paves a path for new biomaterial interfaces, which could be applied in different biomedical fields.

Volume 59, Number 6, November/December 2012, Pages 503–507 •
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Keywords: extracellular synthesis, silver nanoparticles, *Streptomyces albogriseolus*, antibacterial activity, surface plasmon resonance, food pathogens

1. Introduction

In recent years, the development of eco-friendly nanofactories, especially biologically synthesized nanomaterials, has been recognized as a potential means of nanosynthesis [1]. Physical and chemical reduction methods have been conventionally used for the synthesis of metallic nanoparticles. These approaches involve the use of high energy and toxic chemicals in the form of reducing and stabilizing agents. Hence, the use of bio-based approaches for nanosynthesis of noble metals is an utmost need for safer production of nanoparticles [2]. Different biological sources have been used for nanoparticle synthesis. The bioreduction of silver ions by extracellular and intracellular components of bacteria, fungi, and plants has been well documented in much of the literature [3–8].

Silver nanoparticles (AgNPs) are found to have potential applications in various fields because of their strong surface plasmon resonance (SPR). They also exhibit antimicrobial activity [9], antiangiogenic activity [10], antipermeability activity [11], and anticancerous activity [12]. Actinomycetes are a rich source of different bioactive compounds [13]. The genus *Streptomyces* provides nearly 80% of all of the world's antibiotics [14]. The clinically important bacterium *Streptomyces albogriseolus* is a well-known source for the production of novel secondary metabolites. From this strain, a bioactive compound, echinosporin, was isolated, which was found to induce apoptosis and inhibit the cell cycle [15]. Antibiotics, namely toyocamycin, cephamycin C, and neomycin complex, were also produced by this strain [16],[17]. A plant growth-regulating substance, thienodolin, was isolated from the strain [18].

There are few reports on use of actinomycetes such as *Thermomonospora* sp. [19], *Streptomyces hygroscopicus* [20], *Streptomyces parvulus* [21], and *Rhodococcus* sp. [22] for the synthesis of nanoparticles. This work aimed to explore the synthesis of AgNPs using actinobacteria, *S. albogriseolus*, and their role in antibacterial activity. The antibacterial activity of AgNPs was evaluated against three important food

Abbreviations: AgNPs, silver nanoparticles; ISP, International Streptomyces Project; HR-SEM, high-resolution scanning electron microscopy; XRD, X-ray diffraction; TEM, transmission electron microscopy; FTIR, Fourier transform infrared; UV, ultraviolet; SPR, surface plasmon resonance; NCIM, National Collection of Industrial Microorganisms; NCBI, National Center for Biotechnology Information.

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Received 13 June 2012; accepted 18 October 2012
DOI: 10.1002/bab.1054
Published online 18 December 2012 in Wiley Online Library (wileyonlinelibrary.com)

pathogens—*Bacillus cereus*, *Staphylococcus aureus*, and *Escherichia coli*. *B. cereus*, a gram-positive bacterium, causes two different types of food poisoning, a diarrheal type and an emetic type. The diarrheal type can be caused by enterotoxins [23],[24] produced by *B. cereus* in the small intestine [25], whereas the emetic toxin is produced in the food by growing cells of *B. cereus* [26]. *S. aureus* is a cause of gastroenteritis because of consumption of contaminated food [27]. *E. coli*, a gram-negative bacterium, produces heat-stable enterotoxins [28].

To the best of our knowledge, this is the first report on the synthesis and antibacterial activity of AgNPs from *S. albogriseolus*. Ultraviolet–visible (UV–vis) spectroscopy, transmission electron microscopy (TEM), X-ray diffraction (XRD) analysis, and Fourier transform infrared (FTIR) techniques have been used for the characterization of synthesized AgNPs.

2. Materials and methods

2.1. Chemicals

All reagents were of analytical grade and purchased from Sigma–Aldrich (New Delhi, India) and Himedia (Mumbai, India). The culture was grown in liquid medium starch casein broth that contained (g/L) soluble starch, 10 g; potassium nitrate, 2 g; sodium chloride, 2 g; dipotassium hydrogen phosphate, 2 g; $MgSO_4 \cdot 7H_2O$, 0.05 g; calcium carbonate, 0.02 g; $FeSO_4$, 0.01; casein, 0.30 g; pH 7 ± 0.2.

2.2. Isolation and identification of *S. albogriseolus*

S. albogriseolus was isolated from mangrove sediment soil at Pichavaram, Tamil Nadu, India. These soil sediments were collected at a depth of 5–10 cm in sterile plastic bags. All sediment samples were transported to the laboratory in sterile condition and stored at -20°C for further use. One gram wet weight of the soil sediment was suspended in 10 mL of 0.85% sodium chloride. Tenfold serial dilutions were taken and plated on starch casein agar medium. The plates were incubated at 27°C and colonies were purified by subculturing on International Streptomyces Project (ISP)-2 and ISP-3 mediums.

2.3. Molecular level identification by 16S rRNA sequencing

The genotypic characterization was achieved by 16S rRNA sequencing. Phenol chloroform method was used for the isolation of total genomic DNA of the actinobacterial strain. The fluorescent dye terminator method was employed using the sequencing kit for the determination of 16S rRNA nucleotide sequence (ABI Prism Big dye terminator cycle sequencing ready reaction kit v.3.1; EdgeBioSystems, Gaithersburg, MD, USA). The sequenced 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). CLUSTALX software (Plate-Forme de Bio-Informatique, Illkirch Cedex, France) was employed for alignment of the nucleotide sequences. The morphological features of spores were observed by high-resolution scanning electron microscopy (HR-SEM) analysis.

2.4. Extracellular synthesis of AgNPs

The actinomycetes isolate was inoculated on starch casein broth (pH 7.3) for the synthesis of AgNPs. After 5 days' incubation at room temperature, the culture was centrifuged at 2,214*g* for 20 Min and cell-free supernatant was interacted with 2 mM $AgNO_3$ solution. The interacted samples were kept on a rotary shaker for 72 H at room temperature. Silver nitrate (2 mM) and culture supernatant were maintained as controls.

2.5. Characterization of synthesized AgNPs

2.5.1. UV–*vis spectral analysis*

The AgNP formation in the aqueous solution was visually monitored by changes in color. UV–vis spectra were recorded from 300 to 600 nm at regular intervals (6, 12, 24, and 48 H) by using Systronic double beam UV–vis spectrophotometer [UV–vis Systronic-2201 (Systronics, Ahmedabad, Gujarat, India)]. UV–vis spectra were recorded periodically to check the stability of the biosynthesized particles.

2.5.2. Quantification of synthesized AgNPs

The yield of synthesized AgNPs from *S. albogriseolus* was quantified by atomic absorption spectrophotometer (AAS). Synthesized AgNPs were centrifuged at 6,149*g* for 20 Min [29]. The residual amount of silver ions present in the supernatant was estimated by AAS (Varian-AA240). The yield of AgNP production was calculated by using this formula:

$$\text{Conversion of Ag ion} = \left(\frac{\text{Initial concentration of Ag ion (ppm)} - \text{concentration of Ag ion in supernatant (ppm)}}{\text{Initial concentration of Ag ion (ppm)}} \right) \times 100$$

2.5.3. Particle size distribution by dynamic light scattering and TEM analysis

The size distribution of the synthesized AgNPs was determined using particle size analyzer (90 Plus Particle Size Analyzer; Brookhaven Instruments Corporation, Holtsville, NY, USA). A TEM image was taken to confirm the shape and size of the particles [TEM (80 KV); Technai, Philips, FEI Company, Hillsboro,OR, USA]. A small drop of colloidal solution of AgNPs was placed on a carbon-coated copper grid and dried before measurement.

2.5.4. XRD studies

The synthesized AgNP was freeze-dried. The identification of crystalline structure of the AgNPs was determined by XRD analysis (D8 Advance; Bruker, Karlsruhe, Baden-Wurttemberg, Germany). The XRD spectra were recorded between 10° and 100°.

2.5.5. FTIR analysis

The actinobacterial supernatant and the synthesized AgNPs were freeze-dried and used for FTIR analysis by using FTIR spectrometer (Thermo Nicolet Model 6700; Thermo Scientific, Waltham, MA, USA).

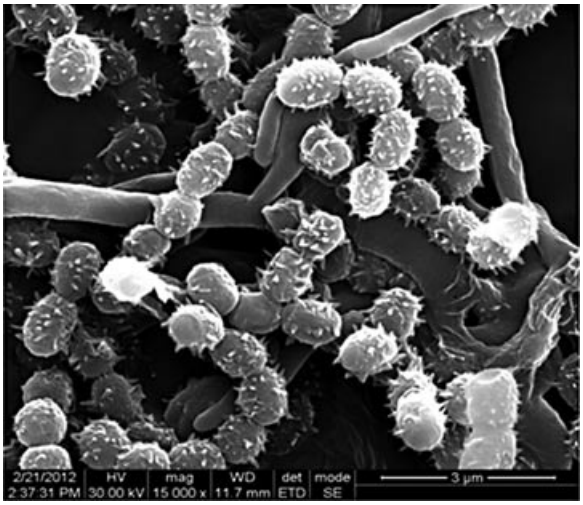

Fig. 1. SEM image of *S. albogriseolus*.

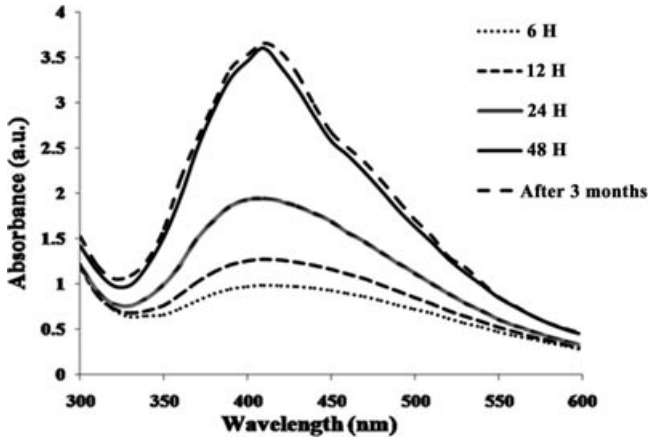

Fig. 2. UV–vis spectra of AgNPs synthesized by extracellular components of *S. albogriseolus*.

2.6. Antibacterial effect of AgNPs by well diffusion method

The antibacterial activity of the synthesized AgNPs was determined by the well diffusion method against *S. aureus* (National Collection of Industrial Microorganisms [NCIM] 2672), *B. cereus* (NCIM 2458), and *E. coli* (NCIM 2809). These pathogenic test organisms were grown in Mueller–Hinton broth (Himedia) at room temperature on a rotary shaker at 130 rpm. Wells of 6-mm diameter were made on the test organism seeded Mueller–Hinton agar plates using gel puncture and each of four wells on all plates was inoculated with 50 µL of synthesized AgNPs, and the rest of the wells were inoculated with controls. An aqueous solution of $AgNO_3$ and the cell-free supernatant were used as controls for this assessment. The diameter of zone of inhibition was measured after 24 H of incubation at room temperature.

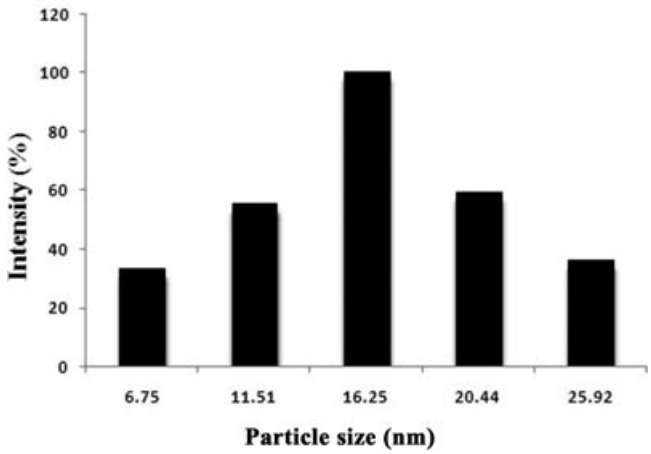

Fig. 3. Size distribution of synthesized AgNPs by DLS analysis.

3. Results and discussion

From the SEM image (Fig. 1), it was found that the spore surface of the actinobacterial strain was spiny and arranged in spirals of continuous chains. The 16S rRNA sequencing analysis identified the isolated strain as belonging to genus *Streptomyces* with a 100% similarity with *S. albogriseolus*. The gene sequence has been submitted to the GenBank under the accession no. JQ682627. The brown coloration of the supernatant containing silver nitrate was observed after 24 H of incubation. The change in color is due to the excitation of the surface plasmon in resonance with the incident light; this phenomenon is known as SPR, which is typical for AgNPs [30]. AgNPs are known to exhibit UV–vis absorption maxima in the range of 400–500 nm because of the SPR property [31]. At the 48th hour, a strong absorption peak was observed around 409 nm (Fig. 2). After 72 H of incubation, there was no change in the intensity, indicating the complete reduction of AgNPs. The synthesis of AgNPs may have occurred because of the presence of proteins and enzymes secreted by *S. albogriseolus*. The synthesized nanoparticles were stable for more than 3 months, which showed no evidence of flocculation. The stability of AgNPs results from the potential barrier that develops as a result of the competition between weak van der Waals forces of attraction and electrostatic repulsion. The AgNPs possess a negative charge due to the adsorption of proteins from the actinobacteria, which help in preventing aggregation by electrostatic stabilization. The production of AgNPs was calculated by AAS. The yield of AgNPs synthesized by *S. albogriseolus* is 72.64%. The size distribution of the synthesized nanoparticles was confirmed from the TEM image and dynamic light scattering (DLS) analysis. The average size of the particle was about 16.25 $\pm$ 1.6 nm and almost spherical in shape (Figs. 3 and 4). The TEM image illustrates the presence of well-distributed AgNPs without any aggregation, indicating that the nanoparticles have been coated by the extracellular components of *S. albogriseolus*. The crystallinity of AgNPs was determined by XRD analysis. The XRD planes obtained at 111, 200, 220, and 311

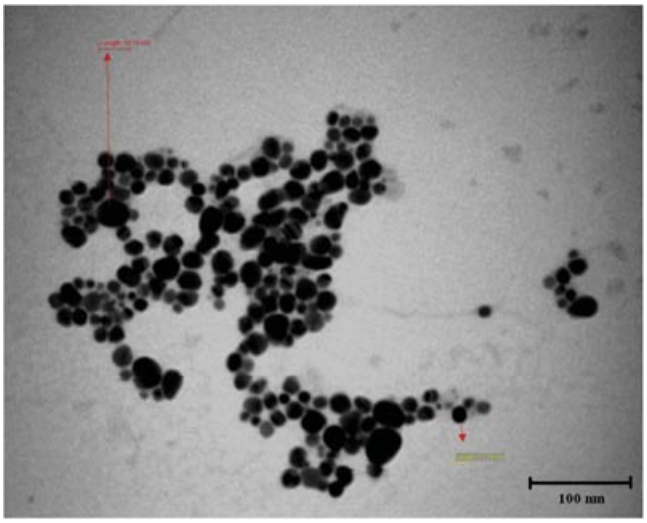

Fig. 4. TEM image of synthesized AgNPs.

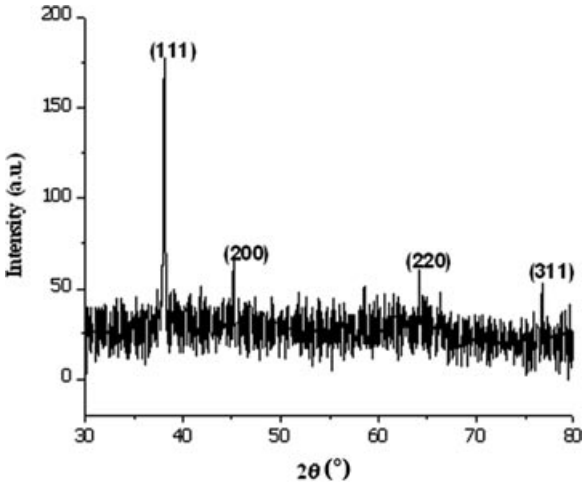

Fig. 5. XRD pattern of synthesized AgNPs.

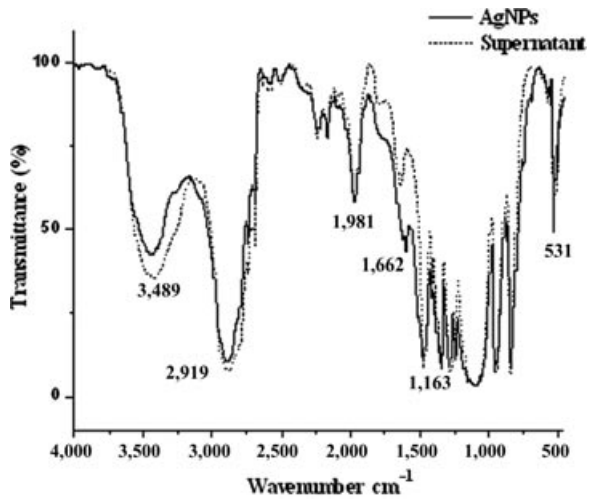

Fig. 6. FTIR spectrum of AgNPs and supernatant of *S. albogriseolus*.

Table 1
Diameter zone of inhibition by AgNPs against food pathogens

Test organism	Zone of inhibition ($n = 3$) (mm in diameter) mean ± SD
Escherichia coli	16.5 ± 0.5
Bacillus cereus	14 ± 1
Staphylococcus aureus	18.5 ± 0.5

Results are mean ± SD ($n = 3$).

points correspond to the cubic face-centers of silver (Fig. 5). The obtained peaks matched those of the database of the Joint Committee on Powder Diffraction Standards (JCPDS) File No. 04–0783 [20].

The FTIR spectrum shows a band around 3,489 cm^{-1} that could be assigned to the N–H symmetric stretching vibrations, which corresponds to the presence of secondary structural components of proteins (Fig. 6) [32],[33]. The band at 1,163 cm^{-1} could be due to the ester C–O asymmetric stretching vibrations. The peak at 2,920 cm^{-1} is characteristic of asymmetric CH_2 stretching [33]. The peak at 531 cm^{-1} may be the out of plane C=O bending. The above-mentioned IR peaks are characteristic of proteins [34]. The bands between 1,980 and 1,950 cm^{-1} are attributed to the C=C=C bonding of polyenes [35]. These polyenes are secreted by marine actinomycetes and they represent the oldest family of antifungal drugs [36],[37]. Hence, it can be concluded that the bioactive compounds containing polyenes and proteins could be the probable capping and stabilizing agents for AgNPs. Similar conclusions were drawn from the FTIR studies by Ahmad et al. [30], Duran et al. [38], and Wei et al. [39], where they reported the role of proteins in the capping and stabilization of AgNPs.

In this study, the AgNPs displayed antimicrobial activity against food pathogens (Table 1, Fig. 7). For each microorganism, the mean diameter of the zone of inhibition for three replicates was determined to be about 14 ± 1, 16.5 ± 0.5, and 18.5 ± 0.5 mm, respectively, for *B. cereus*, *E. coli*, and *S. aureus*. The antibacterial activity was higher against *S. aureus* than against *B. cereus* and *E. coli*. These findings are in agreement with previous studies that examined the antibacterial activity of AgNPs against *E. coli*, *B. cereus*, and *S. aureus* [40]. Silver or silver ions are known to have strong inhibitory and antibacterial effects, as well as a broad spectrum of antimicrobial activities. Generally, silver inhibits the respiration process by binding to the cell membrane and bacterial cell wall [41].

4. Conclusion

Spherical shaped AgNPs of 16.25 ± 1.6 nm size with a yield of 72.64% were synthesized by *S. albogriseolus*, isolated from the mangrove sediment. The FTIR study shows that the

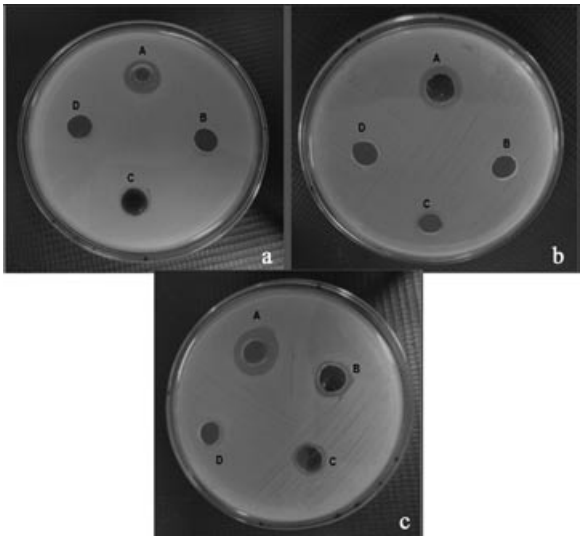

Fig. 7. Antibacterial activity of AgNPs synthesized by *S. albogriseolus* (a) *Escherichia coli*, (b) *Bacillus cereus*, and (c) *Staphylococcus aureus*. In each plate, (A) synthesized AgNPs, (B) control 1—$AgNO_3$, (C) and (D) control 2—culture supernatant.

bioactive compounds containing polyenes and proteins could be the probable capping and stabilizing agents for AgNPs. Therefore, the synthesized nanoparticle was stable for more than 3 months. The biosynthesized AgNPs displayed a pronounced antibacterial activity against the tested food pathogens. This antibacterial activity can be utilized to prevent bacterial colonization and to eliminate microorganisms on food transporting containers to protect and transport food safely without any contamination. However, the elucidation of the exact mechanism behind the nanoparticle synthesis by using microorganisms needs more investigation.

Acknowledgements

The authors are thankful to TANUVAS-Chennai, Sophisticated Analytical Instrumentation Facility, IIT-Chennai, and to the management of VIT University for providing the necessary support.

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