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Streptomyces somaliensis mediated green synthesis of silver nanoparticles

[1]M. Soltani Nejad; [2]M. Khatami; [1]G.H. Shahidi Bonjar*

[1]Department of Plant Pathology, College of Agriculture, Shahid Bahonar University of Kerman, Iran
[2]Department of Biotechnology, College of Agriculture, Shahid Bahonar University of Kerman, Iran

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ABSTRACT:

Objective(s): The development of reliable and ecofriendly process for the synthesis of nano-metals is an important aspect in the field of nanotechnology. Nano-metals are a special group of materials with broad area of applications.

Materials and Methods: In this study, extracellular synthesis of silver nanoparticles (SNPs) performed by use of the gram positive soil Streptomycetes. Streptomycetes isolated from rice fields of Guilan Province, Iran (5 isolates). Initial characterization of SNPs was performed by visual color change. To determine the bacterium taxonomical identity, its colonies characterized morphologically by use of scanning electron microscope. The PCR molecular analysis of active isolate represented its identity partially. In this regard, 16S rDNA of isolate G was amplified using universal bacterial primers FD1 and RP2. The PCR products were purified and sequenced. Sequence analysis of 16S rDNA was then conducted using NCBI GenBank database using BLAST. Also SNPs were characterized by, transmission electron microscopy (TEM) and X-ray diffraction spectroscopy (XRD).

Results: From all 5 collected *Streptomyces somaliensis* isolates, isolate G showed highest extracellular synthesis of SNPs via in vitro. SNPs were formed immediately by the addition of ($AgNO_3$) solution (1 mM). UV-visible spectrophotometry for measuring surface plasmon resonance showed a single absorption peak at 450 nm, which confirmed the presence of SNPs. TEM revealed the extracellular formation of spherical silver nanoparticles in the size range of 5-35 nm.

Conclusion: The biological approach for the synthesis of metal nanoparticles offers an environmentally benign alternative to the traditional chemical and physical synthesis methods. So, a simple, environmentally friendly and cost-effective method has been developed to synthesize SNPs using Streptomycetes.

Keywords: *Biosynthesis, Eco-friendly, 16S rDNA, SNPs, Streptomyces*

INTRODUCTION

Nanotechnology involves the production, manipulation and use of materials which size are less than 100 nm [1] Nanoparticles are a special group of materials with broad area of applications and usually they have large surface area which is chemically more reactive than their fine structural analogues [2,3]. Applications of metallic NPs in the biomedical fields are numerous [4], and there is high potential for continued growth in this area. Metallic NPs are widely used for their antimicrobial functionality; for example, silver NPs (AgNPs) have been incorporated into wound dressings, bone cements and implants [5,6]. In recent years, there has been growing interest in the manufacture of silver nanoparticles. Silver, in its metallic as well as ionic forms, exhibits cytotoxicity against several microorganisms, and hence, is used as an antimicrobial agent [7, 8]. Several physical and chemical methods for synthesis of AgNPs have been developed. In this pursuit, these methods must have control over particle size and usage of non-biodegradable toxic chemicals in some of these methods is probably dangerous to the environment and biological systems [9,10]. Bacterium is always been an organism of choice due to its inherent properties to produce different types of enzymes for chemical detoxification and energy-dependent ion efflux, responsible for reduction and

*Corresponding Author Email: *meysamsoltaninejad@gmail.com*
Tel: (+98) ****
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stabilization of metallic nanoparticles [11].Species of *Streptomyces* are a group of non-motile, gram-positive, filamentous and spore-forming bacteria. Most of *Streptomyces* spp. live in the soil saprophytically and many species have a worldwide distribution [12].The goal of the present research was to optimize the green synthesis of SNPs by *S. somaliensis* isolate G. The biosynth-esized SNPs were characterized using UV-vis spectrophotometer, transmission electron microscope (TEM), and X-ray diffraction spectroscopy (XRD).

MATERIALS AND METHODS

Preparation of microorganisms

Soil samples were collected from rice fields in different localities of Guilan Province in Northern Iran [13]. Several samples rand-omly were selected from the mentioned localities using an open-end soil borer (20 cm in depth, 2.5 cm in diameter) as described previously [14].The soil of the top region (10 cm from the surface) was excluded. Samples were air-dried at room temperature for 10- 15 days and then passed through a 0.8 mm mesh sieve. Samples (10 g) of air-dried soil were mixed with sterile distilled water (100 mL). The mixtures were shaken vigorously for 1h and then allowed to settle for 1h. Portions (1 mL) of soil suspensions (diluted 10^{-1}) were transferred to 9 mL of sterile distilled water and subsequently diluted to 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}. Inocula consisted of adding 1 ml of 10^{-3}-10^{-6} soil dilutions to autoclaved CGA at 50 °C before pouring the 9 cm Petri plates and solidification (15).Three replicates were considered for each. Plates were incubated at 28° C for up to 7 days. From day 3 on, *Streptomyces* colonies were isolated on CGA, incubated at 28° C for two week and stored refrigerated as pure cultures before use. Five strains of *Streptomyces* spp. Isolated. Streptomyces colonies isolated as pure cultures on CGA, grown at 29° C for 14 days and stored at 5° C for next studies.

Biosynthesis of silver nanoparticles

The bacteria, *Streptomyces* spp. were isolated and maintained as pure cultures on casein-glycerol-agar (CGA).

Shaded cultures were grown up in conical flasks containing 100 mL of casein glycerol (CG) medium in a shaker incubator (rpm 130) at 29º C. CG was prepared by mixing the following contents; 3g of casein, 2g of NaCl, 2g of KNO_3, 2g of K_2HPO_4, 0.5g of $MgSO_4$, 0.2 g of $CaCO_3$, 10 g of glycerin in 1000 mL of distilled water. After 10 days of incubation, colonies developed on the medium. After this time, the biomass was prepared for each bacterial isolate.The culture was centrifuged at 4000 rpm for 10 minutes and bacterial biomass (15 gr) collected and used for further experiments. Ten mL of 10^{-3} M aqueous silver nitrate ($AgNO_3$) was added into the biomass (~ 1:1, v/v). Controls consisted mixture of only biomass plus distilled water. The mixtures were then placed in a rotating shaker (100 rpm) at 28° C overnight. After 24 hours, dark brown color formed which was indicative of formation of SNPs. The synthesized SNPs were characterized by UV-vis spectroscopy, transmission electron microscopy (TEM) and X-ray diffraction spectroscopy (XRD).

Identification of the active Streptomyces

From all 5 tested *Streptomyces* isolates, isolate G showed high biosynthesis activity. The colonies were characterized by morphological and phylogenetic analyses. The morphological qualities of isolates G as well as surface ornamentation were evaluated by scanning electron microscopy (SEM) of 15-day-old cultures grown on CGA. Genomic DNA was extracted from cultured cells with GeneAll® Exgene™ Cell Sv kit (http://www.geneall.com). The 16S rDNA of isolates G has been increased by PCR, using universal bacterial primers FD1(52-AGAGTTTGATCCTGGG-32) and RP2 (52 -ACGGCTACCTTGTTACGACTT-32). The samples were put to an initial denaturing step, for 1 min at 94 °C. The thermal profile comprised 30 cycles, consisting of 30 s denaturation at 94 °C, 45 s annealing at 52 °C and 2 min extension at 72 °C (16). The PCR products were purified and sequenced by Macrogen Co, Seol, Korea. Sequence analysis has been done by using BLAST by NCBI (http://www.ncbi.nlm.nih.gov).

RESULTS AND DISCUSSION

Extracellular synthesis of silver nano-particle

The Gram positive soil bacterium *Streptomyces* sp isolate G incubated with silver nitrate solution for 24 h found to be well successful in green biosynthesis of SNPs. Fig. 1(A) shows a test tube containing colorless biomass of isolate G after removal from the culture medium and before immersion in 1 mM $AgNO_3$ solution.

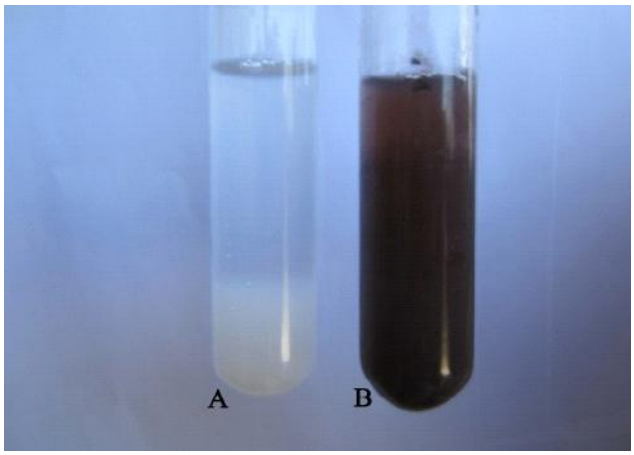

Fig. 1. Biosynthesized silver nanoparticles in a colloidal dispersion by Streptomyces sp isolate G A) colonies before and B) after exposure to $AgNO_3$ after 24 h

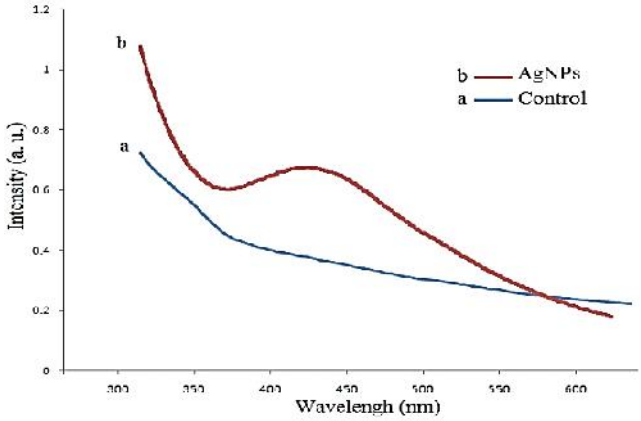

Fig. 2. The UV-Vis spectrometer of silver nanoparticles synthesized by *S. somaliensis* isolate G. After reactions with the *S. somaliensis* isolate G for 24 h. Presence of a strong peak with maximum absorbance at 450 nm is prominent

Color change of the test tube containing the bacterium biomass after immersion in 1 mM $AgNO_3$ solution for 24 hours is shown in Figure 1(B). As indicated, the colorless biomass changed to the deep brown color after 24h of reaction. The appearance of the deep brown color in solution containing the biomass considered as primary indication of the biosynthesis of SNPs in the reaction; however, further analyses approved this assumption.

UV-Vis spectroscopy studies

Both treated and untreated biomasses centrifuged for 5 min at 2000 rpm. Pellets discarded and supernatants used to monitor their UV-Vis absorbance spectra between 300-650 nm wave-lengths. While untreated sample set as reference control, treated sample revealed a prominent peak at 450 nm indicative of presence of SNPs. The spectrum is indicated in Fig 2.

Transmission Electron Microscopy (TEM) analysis

To determine the particle size distribution and shapes of SNPs, TEM was employed. A TEM micrograph recorded from the SNPs shown in Fig 3(A)It is observed that most of the nanoparticles shown in the micrograph are spherical in shape with a size range of 5-35 nm, Fig 3(B)**.**Previously some researchers reported synthesized AgNPs using *Bacillus cereus* in the size ranges 10-30 with and UV-Vis absorbance peak

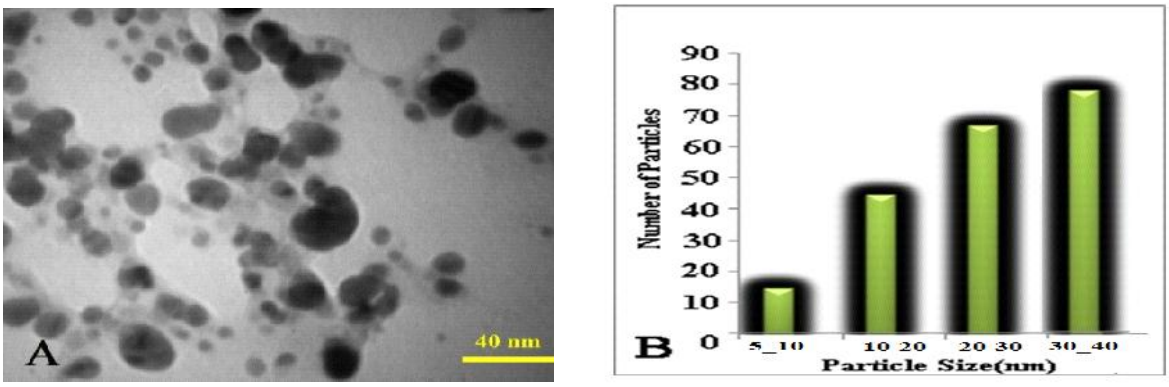

Fig. 3. (A) TEM micrograph recorded of an aqueous solution incubated with *Streptomyces somaliensis* isolate G with Ag^{+} ions for 24 h. (B) Histogram of size distribution of the silver nanoparticles from TEM analysis

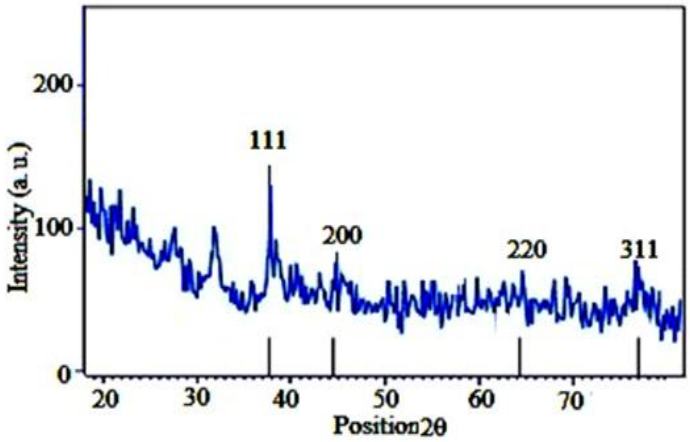

Fig. 4. X-ray diffraction pattern of silver nanoparticles synthesized by *Streptomyces somaliensis* isolate G

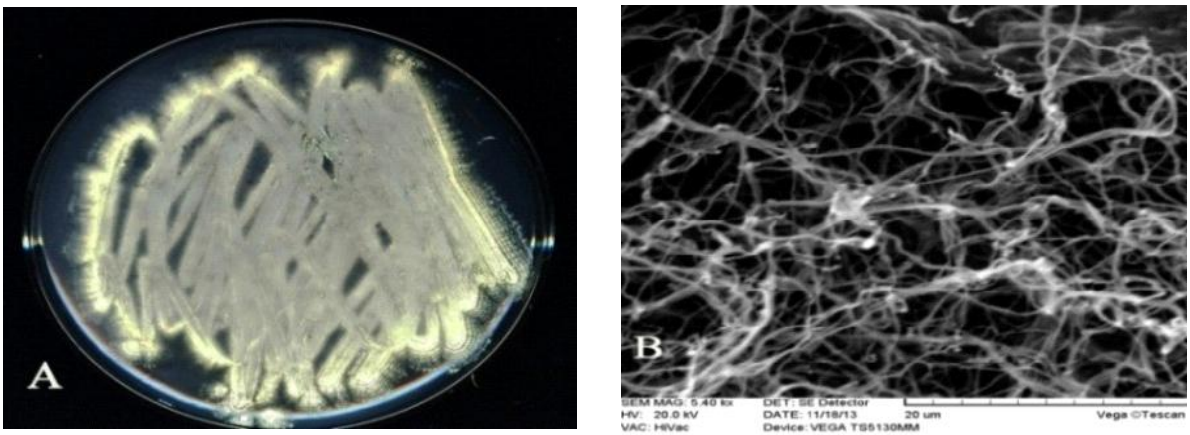

Fig.5. Scanning electron micrograph of *Streptomyces* sp (B) isolate G grown on CGA at 29° C for 10 days (A)

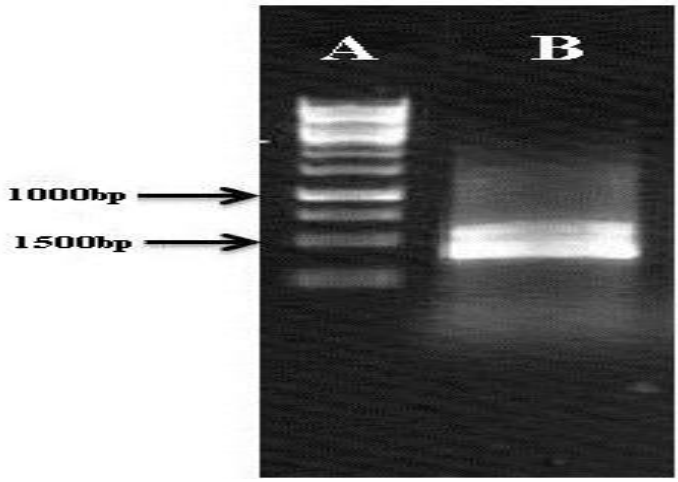

Fig. 6. Amplification of 16S rDNA of *Streptomyces* sp isolate G by PCR (B) and the ladder (A)

at ~ 435 nm [17] and *Aspergillus niger* in the size ranges 1-20 nm and UV-Vis absorbance peak at ~ 440 nm [18].

XRD analysis

Fig 4 shows the X-ray diffraction (XRD) patterns of SNPs biosynthesized by *Streptomyces somaliensis* isolate G. For the crystalline nature of the SNPs, strong XRD peaks were observed about to the (111), (200), (220), (311) planes at 20 angles of 38.28°, 44.38°, 64.54°, and 77.64°, respectively [19].

Scanning Electron Microscopy (SEM) analysis

Streptomyces sp isolate G was grown on CGA (29° C for 10 days) for observation under SEM, Fig. 5(A). Spore chain morphology and spore ornamentation were observed by a scanning electron microscope. Fig 5(B) shows scanning electron micrograph of *S. somaliensis* isolate G..

Molecular identification of Streptomyces somaliensis isolate G

The 16S rDNA of isolate G was amplified by PCR as presented in Fig 6. Comparison of the near full length 16S rDNA sequence of isolate G to GenBank sequences, showed that it was most similar to *S. somaliensis* (E-value = 0.0 and max. identity = 100 %).

CONCLUSION

In this study, we demonstrated the extracellular biosynthesis of SNPs when the biomass of the *S. somaliensis* isolate G was treated by 10^{-3} M aqueous silver nitrate ($AgNO_3$). The SNPs were characterized by UV–Vis spectroscopy, TEM and X-ray diffraction (XRD). The particle sizes were in the range of 5-35 nm. UV–Vis absorbance spectral studies confirmed the strong peak at 450 nm of synthesized AgNPs. The biosynthesis of SNPs using *Streptomyces* sp isolate G is considered an ecofriendly green process.

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