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Research Article

BACILLUS SP. MEDIATED EXTRACELLULAR SYNTHESIS OF SILVER NANOPARTICLES

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ABSTRACT

Objective*:* To synthesize silver nanoparticles extracellularly using *Bacillus sp.* isolated from soil sample.

Methods*:* Bacteria capable of synthesizing silver nanoparticles extracellularly were screened by treating culture supernatant with silver nitrate solution. The potential isolate was isolated on nutrient agar medium and identified by Gram staining and biochemical tests.

Results*:* One of the isolates was found to be potential in extracellular synthesis of silver nanoparticle. The isolate was identified as *Bacillus sp*. The biosynthesized silver nanoparticles were characterized by using UV-Visible spectroscopy, X-ray diffraction analysis, Scanning Electron Microscopy and Atomic Force Microscopic analysis. The biosynthesized silver nanoparticles were found to be spherical with 50-100 nm diameter.

Conclusion*:* Mono-dispersed silver nanoparticles in the range of 50 to 120 nm was synthesised using culture supernatant of newly isolated *Bacillus sp.*

Keywords: Silver nanoparticle, Culture supernatant, Biosynthesis, Extracellular, *Bacillus sp.*

INTRODUCTION

Nanotechnology involves synthesis and application of nanoparticles [1]. Silver nanoparticles are used in wide range of applications as antibacterial agent in disinfecting devices, cosmetics, home appliances and water treatment plants [2,3]. Inorganic composites are used as preservatives in various products [4]. Silver nanoparticles are prepared by physical, chemical and biological methods [5]. The physical and chemical methods are very expensive [6]. Biological method of nanoparticles synthesis would help to remove harsh processing conditions by enabling the synthesis at physiological pH, temperature, pressure, and at the same time at lower cost. Comparison of different synthesis methods gives emphasis to biological synthesis [7]. Biosynthesis of nanoparticles is eco-friendly and has significant advantages over other process [8,9]. Microbial synthesis of nanoparticles can be either intracellular or extracellular [10-14]. Intracellularly synthesized nanoparticles require additional downstream processing steps such as ultrasound treatment, reaction with suitable detergents to release the nanoparticles from cells during their purification [15]. Thus, nanoparticle synthesis by extracellular method is economical with simpler downstream processing as compared to intracellular synthesis. In view of these, the present study focuses on extracellular synthesis of silver nanoparticles from bacterial sources.

MATERIALS AND METHODS

Materials

Silver nitrate was purchased from SD-Fine chemicals, India. Nutrient broth, sodium chloride was purchased from Himedia Labs, India.

Methods

Isolation of Bacillus sp.

Rhizosphere soil sample around carpet grass (*Axonopus compressus*) in the premises of VIT University, Vellore, Tamil Nadu, India was collected in sterile polythene bags and transported to the laboratory. The soil sample was diluted in sterile saline solution (0.9% w/v) and isolates were obtained by spread plate technique on nutrient agar medium at 37°C for 48 h. The morphological and physiological characterization of the isolates was performed according to the methods described in the Bergey's manual of determinative bacteriology.

Extracellular biosynthesis of silver nanoparticles using culture supernatant

Extracellular synthesis of silver nanoparticles were carried out as described by Shahverdi *et al.*[13] with little modifications as given below. The isolated colonies were sub-cultured in nutrient broth and incubated for 24 h at 37°C. The broth was centrifuged at 8000 rpm for 10 min to collect the culture supernatant. 1mM silver nitrate solution was prepared in double distilled water. 200 mL of aqueous solution of 1mM silver nitrate was treated with 100 mL of culture supernatant in a 500 mL Erlenmeyer flask. The whole sample kept in the shaker at 150 rpm and maintained in dark condition. The reduction of silver nitrate was monitored by visible color change of the solution.

Characterization of silver nanoparticles

Primary confirmation of the silver nanoparticle synthesis was carried out by UV-visible spectroscopy in the range of 200-800 nm. The morphology of the silver nanoparticles was examined by FEI Quanta 200 FEG High Resolution Scanning Electron Microscope (HRSEM), Atomic Force Microscopy (AFM) (Hitachi S4800). The chemical composition and crystalline phase of the silver nanoparticles was characterized by Advanced Powder X-Ray diffractometer (D8, Bruker, Germany).

RESULTS AND DISCUSSION

Isolation and identification of the potential isolate

Visible color change of the solution was observed with only one strain. The potential isolate was found to be Gram Positive rod shaped bacteria. Based on biochemical characterization, the isolate was identified as Bacillus sp. (Table 1). The newly isolated Bacillus sp. was used for silver nanoparticle synthesis. The silver nanoparticles were characterized by UV–visible spectroscopy. This technique has proved to be very useful for the analysis of metal nanoparticles. A characteristic broad peak of silver nanoparticles was observed in the UV-visible spectra at 430 nm (Fig.2). It was observed that the peak blue shifted in the absorption spectrum from 400 to 430 nm. The peak formation is due to the collective surface plasmon resonance of electrons at the surface of the silver nanoparticles [16,17]. This shows that biosynthesis of silver nanoparticles using culture supernatant of Bacillus sp. is similar to Enterobacteria and K.pneumonia [13,14].

Table 1: Biochemical characteristics of the isolate capable of synthesizing silver nanoparticle

Characteristics of the potential strain	Results
Gram staining	Positive
Morphology	Rod shaped
Indole	Negative
Methyl red	Negative
Voges proskauer	Positive
Citrate	Positive
Urease	Negative

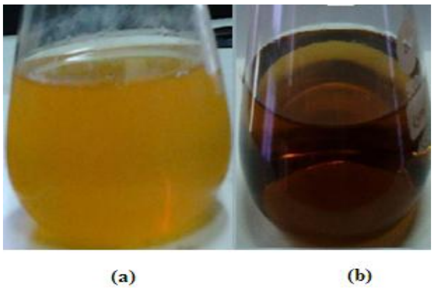


Fig. 1: Color changes during bio-reduction of silver nitrate (a) 0 h (b) after 5 days.

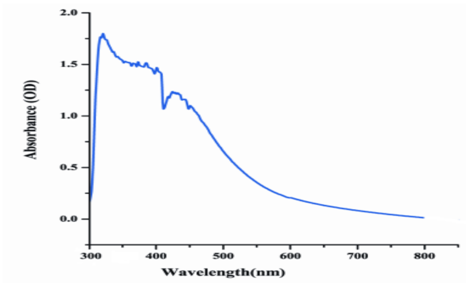


Fig. 2:-UV–Vis absorption spectrum of silver nanoparticles

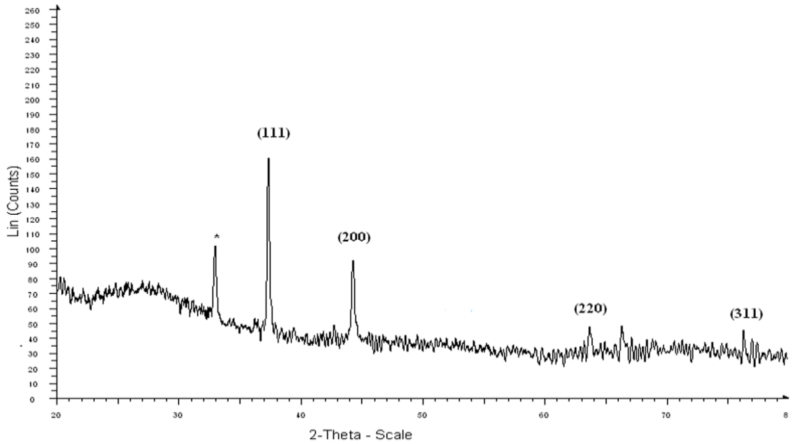


Fig. 3:X-ray diffraction pattern of the silver nanoparticles

Characterization of silver nanoparticles

The culture supernatant + silver nitrate solution changed to yellowish brownish black color indicating the formation of silver nanoparticles (Fig.1).The X-ray diffraction pattern of the synthesized silver nanoparticles was presented in Fig. 3. XRD pattern shows the peak characteristics of metallic silver. The peaks are indexed as the face centered cubic (111), (200), (220) and (311) planes. This indicates the biosynthesized silver nanoparticles are well crystallized [18].

Surface topology of the *Bacillus sp*. mediated synthesized silver nanoparticles was studied by Atomic Force Microscopy analysis (Fig. 4). The micrographs clearly shows that the silver nanoparticle possess spherical shape and did not agglomerate. The calculated sizes are in the range of 80 to 150 nm.

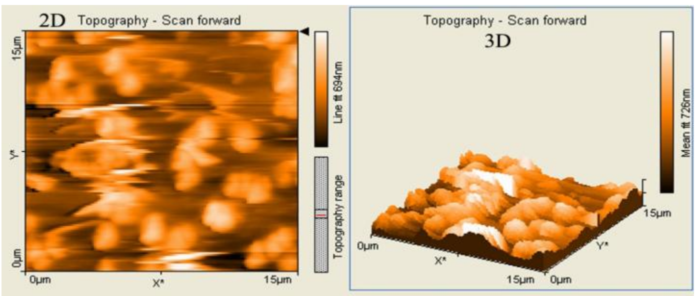
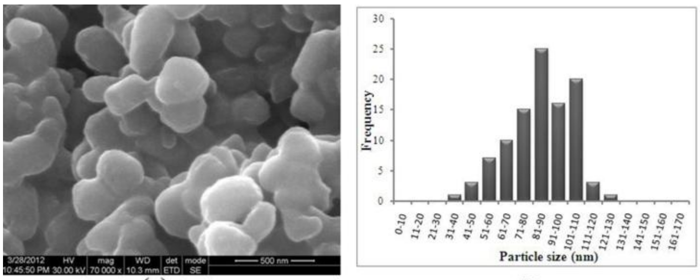


Fig. 4: AFM image of silver nanoparticles



(a) (b)

Fig. 5: (a) SEM image of silver nanoparticles (b) Particle size distribution

HRSEM image confirmed that the silver nanoparticles are almost spherical in morphology with their sizes ranging from 50 to 120 nm (Fig.5a). The average nanoparticle size is about 90 nm (Fig.5b).

CONCLUSION

Biosynthesis of silver nanoparticles using culture supernatant of newly isolated *Bacillus sp.* was demonstrated. Monodispersed silver nanoparticles in the range of 50 to 120 nm was synthesised extracellularly. The extracellular synthesis offers great advantages over intracellular synthesis from the application point of view. In future, it would be important to understand the biochemical and molecular mechanism of the synthesis of the nanoparticles by the cell filtrate. Results conclude that isolated *Bacillus sp.* is a prominent producer of silver nanoparticles.

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REFERENCE

1. Duran N, Priscyla D, Marcato PD, Alves O, De Souza G, Esposito E. Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusarium oxysporum* strains. J Nanobiotechnol 2005;3:1–7.
2. Leaper, DL. Silver dressings: their role in wound management. Int Wound J 2006;3: 282–294.
3. Chopra I. The increasing use of silver-based products as antimicrobial agents: a useful development or a cause for concern? J Antimicrob Chemother 2007;59:587–590.
4. Duran N, Marcarto PD, De Souza, Alves GIH, Esposito OL. Antibacterial effect of silver nanoparticles produced by fungal process on textile fabrics and their effluent treatment. J Biomed Nanotechnol 2007;3:203–208.
5. Roco JC. Trends in nanotechnology patents. Nanopart Res 2005;7:707–712.
6. Li Y, Duan X, Qian Y, Li Y, Liao H. Nano crystalline silver particles: Synthesis, agglomeration, and sputtering induced by electron beam. J Colloid Interface Sci 1999; 209:347-349.
7. Vithiya K, Sen S. Biosynthesis of Nanoparticles. Int J Pharm Sci Res 2011;2:2781-2785.
8. Gade AK, Bonde P, Ingle AP, Marcato PD, Duran N, Rai MK. Exploitation of *Aspergillus niger* for synthesis of silver nanoparticles. J Biobase Mater Bioenergy 2008; 2:243–247.
9. Mukherjee P, Roy M, Mandal BP, Dey GK, Mukherjee PK, Ghatak J, *et al.* Green synthesis of highly stabilized nanocrystalline silver particles by a non-pathogenic and agriculturally important fungus *T. asperellum*. Nanotechnology 2008;19:103–110.
10. Ahmad A, Mukherjee P, Senapati S, Mandal D, Khan MI, Kumar R, *et al*. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. Coll Surf B 2003;28:313–318.
11. Saravanan M, Vemu AK, Barik SK. Rapid biosynthesis of silver nanoparticles from *Bacillus megaterium* (NCIM 2326) and their antibacterial activity on multi drug resistant clinical pathogens. Coll Surf B 2011;88:325–331.
12. Sharma VK, Yngard RA, Lin Y. Silver nanoparticles: Green synthesis and their antimicrobial activities. Adv Colloid Interface Sci 2009;145:83–96.
13. Shahverdi RA, Minaeian S, Shahverdi H, Jamalifar H, Nohi AA. Rapid synthesis of silver nanoparticles using culture supernatants of Enterobacteria: a novel biological approach. Process Biochem 2007;42:919–923.
14. Mokhtari M, Deneshpojouh S, Seyedbagheri S. Biological synthesis of very small silver nanoparticles by culture supernatant of *Klebsiella pneumonia*. The effects of visible-light irradiation and the liquid mixing process. Mater Res Bull 2009;44:1415–1421
15. Kalimuthu K, Babu RS, Venkataraman D, Bilal M, Gurunathan S. Biosynthesis of silver nanocrystals by *Bacillus licheniformis*. Coll Surf B 2008;65:150–153.
16. Henglein A. Physicochemical properties of small metal particles in solution: ''microelectrode'' reactions, chemisorptions, composite metal particles, and the atom-to-metal transition. J Phys Chem B 1993; 97:5457–71.
17. Sastry M, Mayya KS, Bandyopadhyay K. pH Dependent changes in the optical properties of carboxylic acid derivatized silver colloidal particles. Colloid Surf A 1997;127:221–228.
18. Shankar SS, Ahmad A, Pasricha R, Sastry M. Bioreduction of chloroaurate ions by geranium leaves and its endophytic fungus yields gold nanoparticles of different shapes. J Mater Chem 2003;13:1822–1826.