



Characterization and Antimicrobial effect of silver Nanoparticles Synthesized from *Bacillus subtilis* (MTCC 441)

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Abstract:

Researchers in nanotechnology are focusing their research towards the development of silver nanomaterial synthesis as they are known to possess inhibitory and bactericidal effects. Resistance towards bacterial infections has emerged in recent years and is a major health hazard. In the present work attempts were made for the biosynthesis of silver nanoparticles employing the bacteria *B. subtilis* (MTCC 441). The presence of silver nanoparticles was confirmed using sophisticated techniques such as UV-vis spectrophotometer, size and potential of the synthesized silver nanoparticle was measured quantitatively using Zetasizer. The morphology and uniformity of the particle was determined using Transmission Electron Microscope (TEM). The Protein and silver nanoparticles interaction was identified using Fourier Transform Infrared Spectroscopy (FTIR). Synthesized nanoparticle was also evaluated for its antimicrobial efficacy against *S. aureus*, *P. aeruginosa*, *E. coli* and *C. albicans*. The highest antimicrobial efficacy was observed for *C. albicans* and *S. aureus*. This is the first report on the synthesis and antimicrobial efficacy of silver nanoparticle developed from *B. subtilis* (MTCC 441).

Keywords: Silver nanoparticles; *B. subtilis* (MTCC 441); FTIR; TEM; Antimicrobial efficacy.

1. Introduction

Due to its wider applications in the field of sensors, biolabeling and development of novel antimicrobial agents in the synthesis of silver nanoparticles is an emerging area of research in the nanotechnology [1]. Researchers show a great deal of interest towards the biosynthesis of metallic nanoparticles involving various microorganisms [2], [3], [4], [5], [6], [7] and [8]. Intracellular synthesis of nanoparticles by bacteria, fungi and yeast is highly expensive as compared to extracellular synthesis [9], [10] and [11].

Development of strains with reduced susceptibility to antibiotics is continuously increasing today at an alarming rate and has become a serious health problem [12] and [13]. Environmental microorganisms, such as bacteria, molds, yeasts and viruses were often pathogenic and cause severe infections in human beings hence there is a need to search and develop a new antimicrobial agents from natural and inorganic substances [14]. Silver ions possess broad spectrum antimicrobial property and are particularly useful in polymicrobial colonization which is associated with biomaterial related infections [15]. Nanoparticles exhibit new properties compared

to larger particles of the bulk material and these novel properties are derived due to the specific characteristics variation of the particles such as size, distribution and morphology [16]. Nanoparticles possess a higher surface area-to-volume ratio with decrease in the size of the particles [17], [18] and [19]. The important criterion in the silver nanoparticle biosynthesis is size control. Among Metal nanoparticles, silver nanoparticles are recognized as high inhibitory agent against microbes with broad range of target sites both intracellular as well as an extracellular at low concentrations and with no side effects [20].

In the present investigation, attempts were made for the synthesis of silver nanoparticles using the bacterium *B. subtilis* (MTCC 441). In the present study synthesis and formation of silver nanoparticles was confirmed by UV-vis spectrophotometer, size and potential was determined using Zetasizer, morphology by TEM (Transmission electron microscopy) and understanding of protein-silver nanoparticles interaction by employing Fourier transform infrared spectroscopy (FTIR). From the literature survey it is noticed that no study was reported using this bacterial strain for synthesizing silver nanoparticles. In this paper the synthesis of silver nanoparticles is reported by simultaneous reduction of aqueous Ag⁺ with the culture broth of *B. subtilis* (MTCC 441). Further the biologically synthesized silver nanoparticle was subjected to antimicrobial efficacy studies and it is observed that, the synthesized silver nanoparticle revealed high activity against tested pathogens.

2. Materials and methods

2.1. Synthesis of silver nanoparticles

The bacterium *B. subtilis* (MTCC 441) was grown in a freshly prepared, sterilized nutrient broth and incubated at 37°C for 24 h. After the incubation time the culture was centrifuged at 12,000 rpm and the supernatant was used for the synthesis of Ag-NPs. *B. subtilis* (MTCC 441) culture supernatant was separately added to the reaction vessels containing silver nitrate solution (1 mM) and incubated at room temperature until the color changes were observed.

2.2. Characterization of silver nanoparticles

2.2.1. UV-vis spectrophotometer

The reduction of pure silver ions was monitored with the help of UV-vis spectra and the absorbance was recorded at 200-800 nm range using UV-vis spectrophotometer (Lambda-25; PerkinElmer).

2.2.2. Zetasizer

The size and potential of silver nanoparticles were characterized by Zetasizer (Malvern Zetasizer Nano-ZS, Malvern Instruments, and UK).

2.2.3. TEM

Transmission Electron Microscopy (JEM 2100F, JEOL, The Japan) was used to determine the shape and size range.

2.2.4. FTIR

The FTIR spectrum of the sample was obtained using a FTIR spectrophotometer (Perkin Elmer, USA).

2.3. Assay for antimicrobial activity of silver nanoparticles

The antimicrobial efficacy of silver nanoparticles was evaluated against *S. aureus*, *P. aeruginosa*, *E. coli*, and *C. albicans* employing agar well diffusion method. Approximately 10⁷ colony-forming units of each microorganism were inoculated on Muller Hinton agar (MHA) plates and 50 µL of silver nanoparticles were added into each well using a

sterile cork borer. The bacteria inoculated plates were incubated at 37 °C for 24 h and yeast inoculated plates were incubated at room temperature for 2 days. After incubation period, the zone of inhibition was observed and tabulated.

3. Results and discussion

The *B. subtilis* (MTCC 441) cell filtrate present in reaction vessels got converted to brown color from transparent color after treatment with silver nitrate solution (1mM). The brown color appearance indicates the formation of silver nanoparticles [21] and [22].

3.1. Characterization of silver nanoparticles by UV-visible spectroscopy

The above solution was subjected to optical measurements by UV-vis Spectrophotometer (Fig.1). A peak around 425 nm wavelength suggested the presence of silver nanoparticles in the solution. This is the specific wavelength which indicates synthesized silver nanoparticles [23]. The occurrence of peak at absorption intensity between 400-450 nm indicated the presence of surface plasmon, which reflected the presence of silver nanoparticles with size ranging from 2 nm to 100 nm [24]. The absorbance peak reflects the size and shape of the silver nanoparticles formed [25] and [26].

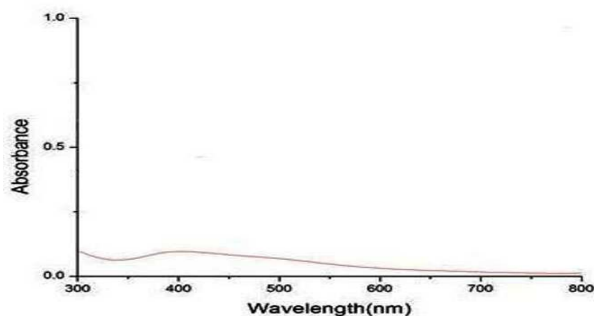


Fig.1. UV-VIS absorption spectrum of synthesized silver nanoparticles

3.2. Characterization of silver nanoparticles by Zetasizer

The size and potential of the synthesized silver nanoparticles were determined using Zetasizer [27]. It was observed that the biologically synthesized silver nanoparticles possessed size of 232 nm and -21.8 mV potential (Fig.2 and Fig.3).

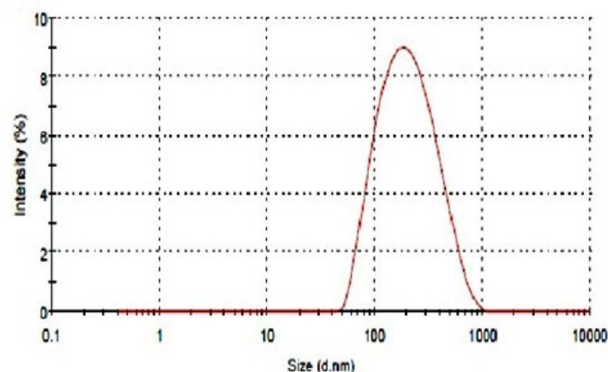


Fig. 2: zeta size of synthesized silver nanoparticles from *B. subtilis* (MTCC 441)

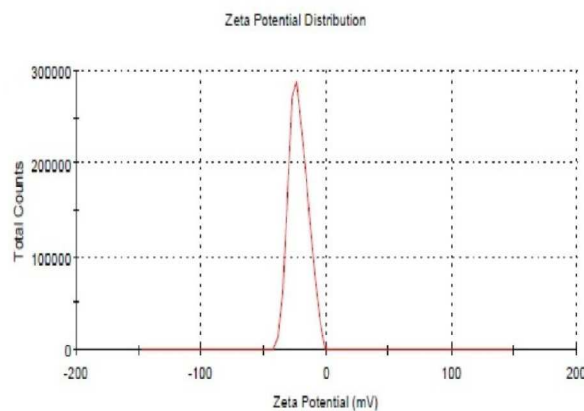


Fig.3: zeta potential of synthesized silver nanoparticles *B. subtilis* (MTCC 441)

3.3. Characterization of silver nanoparticles by TEM

The morphology and the size range of synthesized silver nanoparticles were detected using the cell-free culture supernatants of the bacterial strains by employing TEM. The synthesized silver nanoparticles had a spherical shape and size ranging from 10–100 nm (Fig.4). The silver nanoparticles produced were more distinct and were scattered in their distribution [28].

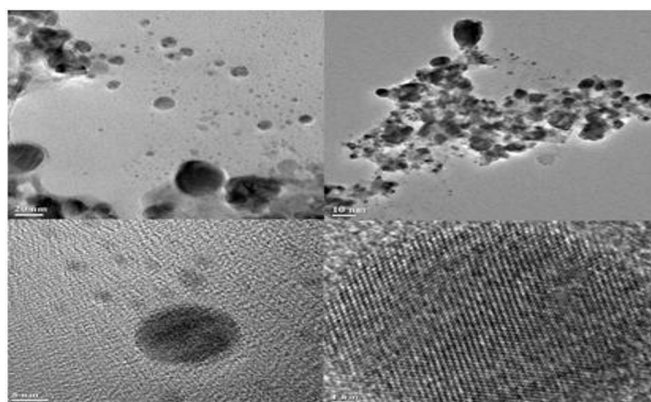


Fig.4: TEM micrograph showing synthesized silver nanoparticles of various size ranges 10–100 nm.

3.4. Characterization of silver nanoparticles by FTIR

The FTIR was used to identify possible interactions between the Ag salts and protein molecules, which could account for the reduction of silver ions and the stabilization of silver nanoparticles (Fig.5). The spectra revealed three bands at

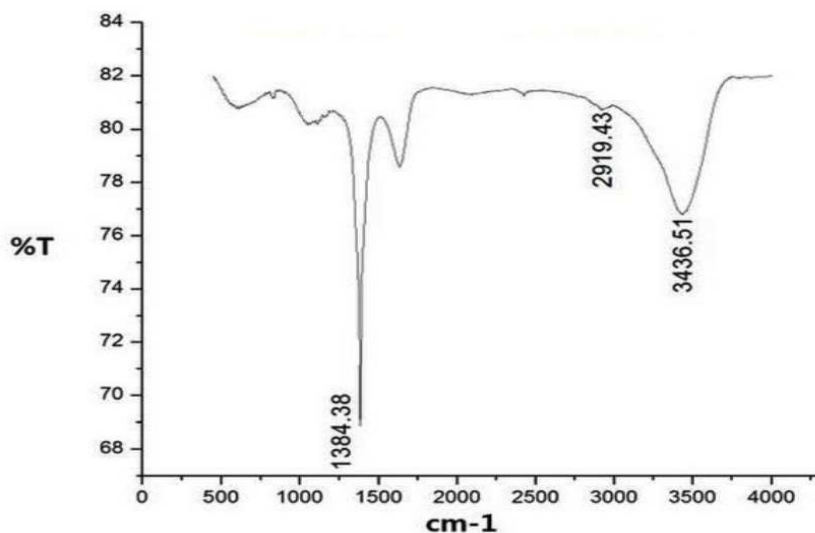


Fig.5. FTIR spectrum showing peaks at 3436.51, 2919.43 and 1384.38 cm^{-1} .

The antimicrobial efficacy of synthesized silver nanoparticles were investigated against various pathogenic organisms such as *S. aureus*, *P. aeruginosa*, *E. coli*, and *C. albicans* using agar well diffusion method. The diameter of inhibition zones (mm) around each well with silver nanoparticles solution and positive control is represented in (Table 1). The silver nanoparticles

1384.38 cm^{-1} , 3436.51 cm^{-1} and 2919.43 cm^{-1} for silver nanoparticles in solution. The presence of the amide linkages between amino acid residues in the polypeptides provides a well known signature in the infrared region of the electromagnetic spectrum. The bands at 1384.38 cm^{-1} correspond to C-N stretching vibration. The bands at 3436.51 cm^{-1} and 2919.43 cm^{-1} were assigned to the stretching vibrations of primary and secondary amines. The overall observation thus confirmed the presence of protein in the samples of silver nanoparticles. It is well known that proteins can bind to the silver nanoparticles either through free amine groups or cysteine residues in the proteins [29]. FTIR spectroscopy thus revealed the possible stabilization of silver nanoparticles with proteins.

3.5. Antibacterial activity

were found to possess high antimicrobial activity against *C. albicans* (19mm) and *S. aureus* (15mm) respectively and the lesser antimicrobial activity of silver nanoparticles was found against *P. aeruginosa* (9mm) and *E. coli* (5mm). The silver nanoparticles showed high antimicrobial property due to their large surface area, which makes better contact with microorganisms. The

nanoparticles get attached to the cell membrane and also penetrated inside the bacteria. The bacterial membrane is known for its sulfur-containing proteins, these might be the preferential sites for the silver nanoparticles to penetrate. On the other hand, nanoparticles found inside will also tend to react with other sulfur-containing proteins in the interior of the cell, as well as with phosphorus-containing compounds such as DNA. To conclude, the changes in morphology of bacterial membrane as well as the possible damage caused by the nanoparticles reacting with the DNA will affect the bacteria in cell processes such as the respiratory chain and cell division, finally causing cell death. Further nanoparticles might also release silver ions in the bacterial cells, which further enhance their bactericidal activity [30] and [31].

Table 1: Inhibitory activities of synthesized silver nanoparticles

S. No.	Pathogens	Zone of inhibition (mm)
1.	<i>S. aureus</i>	15
2.	<i>P. aeruginosa</i>	9
3.	<i>E. coli</i>	5
4.	<i>C. albicans</i>	19

4. Conclusion

In this study, attempts were made for the synthesis of silver nanoparticles from the microbe *B. subtilis* (MTCC 441) and approaches were also made to evaluate their antimicrobial potentials. In the present work, synthesis and formation of silver nanoparticles was confirmed through analytical tools like UV-vis spectrophotometer, Zetasizer, TEM and FTIR. The antimicrobial efficacy of synthesized silver nanoparticle was well demonstrated by the formation of a clear zone of

inhibition against *S. aureus*, *P. aeruginosa*, *E. coli* and *C. albicans*. Thus the biologically synthesized silver nanoparticles could find immense use in medical field particularly in the development of novel antimicrobial agents due to its antimicrobial potentials.

Reference

- 1) Jaidev LR, Narasimha G. Fungal mediated biosynthesis of silver nanoparticles, characterization and antimicrobial activity. *Colloids and Surfaces B: Biointerfaces*. 2010; 81: 430-433.
- 2) Ahmad A, Senapati S, Khan MI, Kumar R, Sastry M. Extracellular biosynthesis of monodisperse gold nanoparticles by a novel extremophilic actinomycete, *Thermomonospora* sp. *Langmuir*. 2003; 19: 3550-3553.
- 3) Mandal D, Bolander ME, Mukhopadhyay D, Sankar G, Mukherjee P. The use of microorganisms for the formation of metal nanoparticles and their application. *Applied Microbiology and Biotechnology*. 2006; 69: 485-492.
- 4) Basavaraja S, Balaji SD, Lagashetty A, Rajaab AH, Venkataraman A. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium semitectum*". *Materials Research Bulletin*. 2008; 43: 1164-1170.
- 5) Vigneshwaran N, Ashtaputre NM, Varadarajan PV, Nachane RP, Paralikar KM, Balasubramanya RH. Biological synthesis of silver nanoparticles using the fungus *Aspergillus flavus*. *Materials Letters*. 2007; 61: 1413-1418.
- 6) Vigneshwaran N, Kathe AA, Varadarajan PV, Nachane RP, Balasubramanya RH. Biomimetics of silver nanoparticles by white rot fungus, *Phaenerochaete chrysosporium*. *Colloids and Surfaces B: Biointerfaces*. 2006; 53: 55-59.
- 7) Shahverdi AR, Minaeian S, Shahverdi HR, Jamalifar H, Nohi V. Rapid synthesis of silver

- nanoparticles using culture supernatants of Enterobacteria: A novel biological approach. *Process Biochemistry*. 2007; 42: 919-923.
- 8) Shahverdi AR, Fakhimi A, Shahverdi HR, Minaian S. Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against *Staphylococcus aureus* and *Escherichia coli*. *Nanomedicine: Nanotechnology, Biology and Medicine*. 2007; 3: 168-171.
 - 9) Klaus T, Joerger R, Olsson E, Granqvist CG. Silver-based crystalline nanoparticles, microbially fabricated. *Proceedings of the National Academy of Sciences*. 1999; 96: 13611-13614.
 - 10) Kowshik MA, Ashtaputre S, Kharrazi S, Vogel W, Urban J, Kulkarni SK, Paknikar KM. Extracellular synthesis of silver nanoparticles by a silver-tolerant yeast strain MKY3. *Nanotechnology*. 2003; 14: 95-100.
 - 11) Mukherjee P, Ahmad A, Mandal D, Senapati S, Sainkar SR, Khan MI, Ramani R, Parischa R, Ajayakumar PV, Alam M, Sastry M, Kumar R. Bioreduction of AuCl₄(-)⁻ Ions by the Fungus, *Verticillium* sp. and Surface Trapping of the Gold Nanoparticles Formed. *Angewandte Chemie International Edition*. 2001; 40: 3585-3588.
 - 12) Wright GD. Resisting resistance: new chemical strategies for battling superbugs. *Chemistry & Biology*. 2000; 7: 27-32.
 - 13) Wright GD. Bacterial resistance to antibiotics: enzymatic degradation and modification. *Advanced Drug Delivery Reviews*. 2005; 57: 1451-70.
 - 14) Kim T N, Feng QL, Kim JO, Wu J, Wang H, Chen GC, Cui FZ. Antimicrobial effects of metal ions (Ag⁺, Cu²⁺, Zn²⁺) in hydroxyapatite. *Journal of Materials Science: Materials in Medicine*. 1998; 9: 129-134.
 - 15) Gristina AG. Biomaterial-centered infection: Microbial adhesion versus tissue integration. *Science*. 1987; 237: 1588-95.
 - 16) Savithamma N, Linga Rao M, Rukmini K, Suvarnalatha devi P. Antimicrobial activity of Silver Nanoparticles synthesized by using Medicinal Plants. *International Journal of ChemTech Research*. 2011; 3: 1394-1402.
 - 17) Gupta A, Silver S. Silver as a biocide: will resistance become a problem?. *Nature Biotechnology*. 1998; 16: 888.
 - 18) Kurihara K, Rockstuhl C, Nakano T, Arai T, Tominaga J. The size control of silver nanoparticles in SiO₂ matrix film. *Nanotechnology*. 2005; 16: 1565-1568.
 - 19) Pal S, Tak YK, Song JM. Does the Antibacterial Activity of Silver Nanoparticles Depend on the Shape of the Nanoparticle? A Study of the Gram-Negative Bacterium *Escherichia coli*. *Applied and Environmental Microbiology*. 2007; 73: 1712-1720.
 - 20) Cho KH, Park JE, Osaka T, Park SG. The study of antimicrobial activity and preservative effects of nanosilver ingredient. *Electrochimica Acta*. 2005; 51: 956-960.
 - 21) Sastry M, Ahmad A, Islam Khan M, Kumar R. Biosynthesis of Metal Nanoparticles using Fungi and Actinomycete. *Current Science*. 2003; 85: 162-170.
 - 22) Mulvaney P. Surface plasmon spectroscopy of nanosized metal particles. *Langmuir*. 1996; 12: 788-800.
 - 23) Shankar SS, Absar A, Murali S. Geranium Leaf Assisted Biosynthesis of Silver Nanoparticles. *Biotechnology Progress*. 2003; 19: 1627-1631.
 - 24) Henglein A. Physicochemical properties of small metal particles in solution: "Microelectrode" reactions, chemisorption, composite metal particles, and the atom-to-metal transition. *Journal of Physical Chemistry*. 1993; 97: 5457-5471.
 - 25) Kerker M. The optics of colloidal silver: something old and something new. *Journal of Colloid and Interface Science*. 1985; 105: 297-314.
 - 26) Sosa IO, Noguez C, Barrera RG. Optical properties of metal nanoparticles with arbitrary shapes. *Journal of Physical Chemistry B*. 2003; 107: 6269-6275.

- 27) Amit Kumar Mittal, Abhishek Kaler, Uttam Chand Banerjee. Free radical scavenging and antioxidant activity of silver nanoparticles synthesized from flower extract of *Rhododendron dauricum*. Nano Biomedicine and Engineering. 2012; 41: 18-124
- 28) Mock JJ, Barbic M, Smith DR. Shape effects in plasmon resonance of individual colloidal silver nanoparticles. Journal of Chemical Physics. 2002; 116: 6755-6758.
- 29) Gole A, Dash C, Ramakrishnan V, Sainkar V, Mandale AB, Rao M. "Pepsin-Gold Colloid Conjugates: Preparation, Characterization, and Enzymatic Activity. Langmuir. 2001; 17: 1674-1679.
- 30) Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria. Journal of Colloid and Interface Science. 2004; 275: 177-182.
- 31) Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramfrez JT, Yacaman M. The bactericidal effect of silver nanoparticles. J. Nanotechnology. 2005; 16: 2346-2353.

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