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Antibacterial properties of silver nanoparticles synthesized by *Bacillus megaterium*

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Abstract
In present work silver nanoparticles (AgNps) were synthesized intracellular by using *Bacillus megaterium sp.* Biomass of *Bacillus megaterium* were added into the reaction vessel in which 100 ml of silver nitrate (1 mM) solution. The silver nanoparticles were characterized by Transmission Electron Microscopy (TEM), UV-Visible Spectroscopy (UV) And Fourier Transform Infrared Spectroscopy (FTIR). Antibacterial activity was studied against the human pathogenic microorganism such as *Escherichia coli, Staphylococcus aureus, Pseudomonas aeureginosa, and Proteus vulgaris.*

Keywords: biosynthesis, *bacillus megaterium*, silver nanoparticles, TEM

Introduction
An important area of research in nanotechnology is the biosynthesis of nanoparticles such as nanosilver. Biologically synthesized silver nanoparticles could have many applications, such as spectrally selective coatings for solar energy absorption and intercalation material for electrical batteries [1] as optical receptors, [2] catalysts in chemical reactions, biolabelling, [3] etc. As a result, researchers in the field of nanoparticle synthesis and assembly have turned to biological systems for inspiration [4-5]. It has been known for a long time that in nature a variety of nanomaterials are synthesized by biological processes. For example, the magnetotactic bacteria synthesize intracellular magnetite or greigite nanocrystallites, [6] the other examples are diatomas, which synthesize siliceous materials, [7-8] and S-layer bacteria that produce gypsum and calcium carbonate layers [9-10].
Among inorganic antimicrobial agents, silver has been employed most widely since ancient times to fight infections. The antibacterial and antiviral activity of silver, silver ions, and silver compounds has been thoroughly investigated [11-16]. A survey of recent literature showed remarkable findings on the bactericidal activity of silver nanoparticles (Ag-NPs) [15, 16]. Ingle and co-workers [17], found that Ag-NPs exhibited significant antimicrobial activity against *Escherichia coli* and multi drug resistant *Staphylococcus aureus*, and Pal *et al.* [18] reported that the antibacterial activity of Ag-NPs against the gram-negative *Escherichia coli* depends on the shape of the nanoparticles.
In high concentration, silver is toxic to human beings, whereas in low concentrations it is nontoxic [18]. Metal nanoparticles have been studied extensively because of their exclusive catalytic, optical, electronic, magnetic, and antimicrobial properties [16, 19]. Recent studies have confirmed that specially formulated metal nanoparticles have good antibacterial activity [20] and that nanoparticles-based antimicrobial formulations could be effective bactericidal materials.
These results showed that microorganisms could indeed be used for the intracellular synthesis of nanoparticles. However, the biosynthesis of silver nanoparticles by free cell system and culture filtrate has not been investigated yet. In this paper, we report on the synthesis of silver nanoparticles by the reduction of aqueous Ag+ ion by simultaneous reduction of aqueous Ag+ with the culture broth of some tested *Bacillus megaterium*.

Materials and Methods
Chemicals
Preparation of nutrient broth 100 ml which contains beef extract (0.15 gm), bacteriological peptone (0.5 gm), yeast extracts (0.15 gm) sodium chloride (0.5 gm).

Bacteria
Bacillus megaterium (MTCC 428), *Escherichia coli* (MTCC 739), *Staphylococcus aureus* (MTCC 96), *Pseudomonas aeureginosa* (MTCC 424), *Proteus vulgaris* (MTCC 426) were procured from the Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India.

Synthesis of AgNps
The *Bacillus megaterium* was grown in 250-mL Erlenmeyer flasks containing 100 ml Nutrient broth at 37° C and 150 rpm for 24 hours. After incubation, biomass was separated by centrifugation and washed with sterile distilled water to remove the traces of media components, and, challenged with AgNO3 solution (1 mM). Incubate the solution for 48 hr and after that centrifuged the solution and separate out the biomass and supernatant solution.

Characterization of Ag-NPs:
After 48 hours of incubation of the above mixture, the preliminary detection of Ag-NPs was carried out by visual observation of color change of the cell filtrate. These samples were later subjected to optical measurements, which were carried out by using a UV-Vis spectrophotometer (Shimadzu 1650 PC) and scanning the spectra between 430 nm at the resolution of 1 nm. In Fourier transform infrared (FTIR)

analysis, the FTIR spectrum of the dried sample was recorded on a Shimadzu 8400s instrument in the range 750 to 4000 cm–1 at a resolution of 1 cm^{-1}. A Transmission electron microscopy (TEM) was used to record the micrograph images of synthesized Ag-NPs.

Agar well diffusion assay to evaluate combined effects

Agar well diffusion method was used to evaluate in vitro antibacterial activity of biosynthesized silver nanoparticles against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeureginosa, and Proteus vulgaris* on Nutrient agar media. Bacterial cell filtrate used for the synthesis of silver nanoparticles was used as negative control. These plates were then incubated at 37°C for 24 hours. After incubation, the zones of inhibition were measured.

Results and Discussion

Bacillus megaterium is selected for the synthesis of silver nanoparticles because of very few reports were shows that the synthesis of silver nanoparticles by using *bacillus sp.* In present study biosynthesis of silver nanoparticles by using *Bacillus megaterium* and antibacterial activity on *Escherichia coli* (MTCC 739), *Staphylococcus aureus* (MTCC 96), *Pseudomonas aeureginosa* (MTCC 424), *and Proteus vulgaris* (MTCC 426). The conical flask with *Bacillus megaterium* strain biomass were a pale yellow color before the addition of Ag ions and this change to a brown color clearly indicates the formation of silver nanoparticles in the reaction mixture. Characteristic brown color due to the excitation of Plasmon vibrations in the nanoparticles and provides a convenient signature of their formation. The formation of brown color suggests the presence of silver nanoparticles.

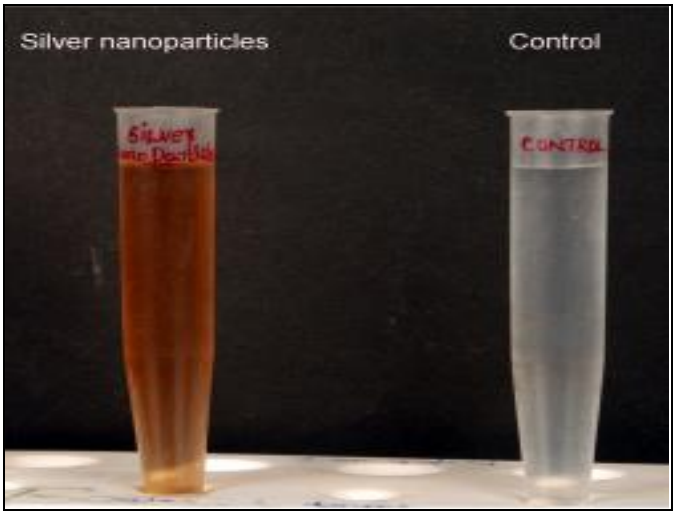

Fig 1: A - Brown color appears to the presence of silver nanoparticles and B - Control Sample)

UV-Visible Spectroscopy

The biomass were mixed with 10^{-3} M silver nitrate of final concentration and incubate for 48 hr. Synthesized silver nanoparticles were characterized by UV-Vis spectroscopy, a strong broad surface Plasmon peak located at 430 nm.

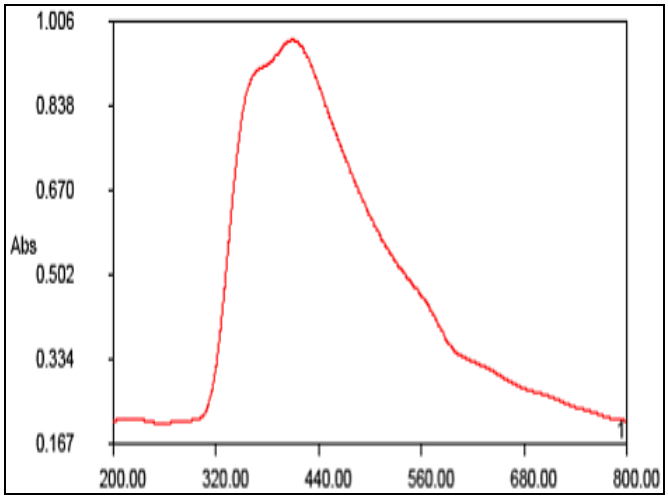

Fig 2: Absorption Spectra of silver nanoparticles by UV-Visible Spectroscopy)

Transmission Electron Microscopy (TEM)

The solution was extremely stable even for several weeks after reaction, it is known that silver cations area highly reactive and tend binds strongly to electron donor groups contains sulphur, oxygen or nitrogen. When we challenged the cell biomass of *Bacillus megaterium* it was observed that the silver was reduced intracellular and extracellular by *Bacillus megaterium*. The assay of silver nanoparticles was performed by TEM micrograph at 1,000,000x times magnification was shown in (fig2). In this micrograph observed spherical nanoparticles in the size range of 6 to 50 nm.

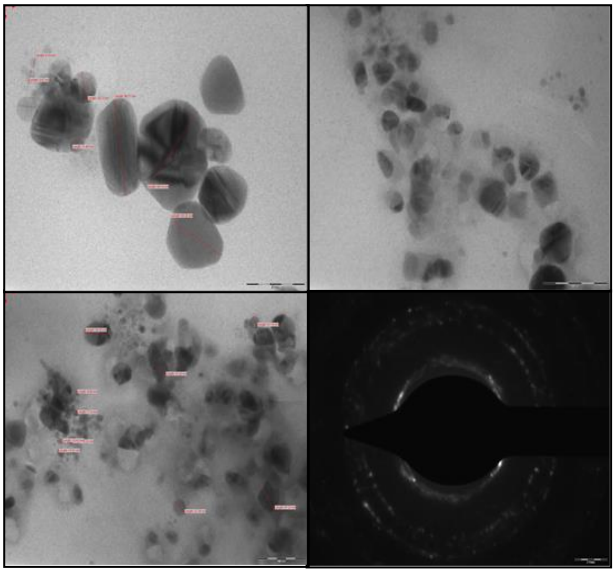

Fig 3: TEM Images of silver nanoparticles of *Bacillus megaterium sp.*

Fourier Transform Infra Red Spectroscopy

FTIR measurements of the dried and powdered samples of Ag-NPs showed the presence of bands 3090.07 - NH stretching, 1554.68 - CN Stretching, NH bending, 1631.83 - C=O Stretching, 763.84 - out of plane NH bending.

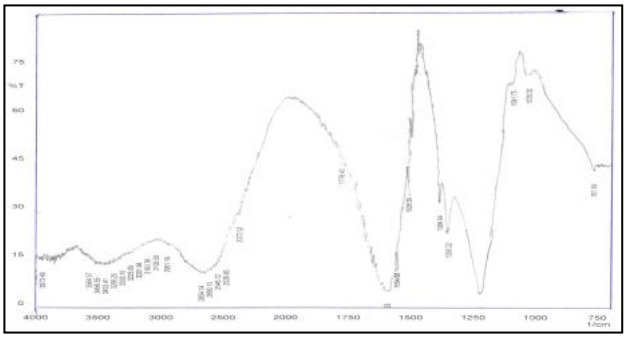

Fig 2: FTIR Spectrum of Silver Nanoparticles of *Bacillus megaterium*

Antibacterial Activity Test for AgNPs

In vitro antibacterial activity of the samples was evaluated by agar well diffusion method with nutrient agar and a determination of inhibition zone in millimeters (mm) which confirms to the recommended standards of the National Committee of Clinical Laboratory Standard. The antibacterial activity against Gram Negative Bacteria, *Escherichia coli* (MTCC 739) and *Pseudomonas aeureginosa* (MTCC 424) and Gram positive bacteria *Staphylococcus aureus* (MTCC 96) and *Proteus vulgaris* (MTCC 426) at different concentration of AgNPs. In order to recover the lyophilized culture, the desired culture contained was aseptically transferred into the nutrient broth and maintained in an incubator at 37^{o}C for 3 h. The bacterial culture was spread aseptically into the solid agar plates. After spreading there was prepare well which were dipped into the samples of AgNPs at different concentration. And lastly the solid agar plates were incubating at 37^{0}C for 24 hrs. The diameters of inhibition zone around the samples were used to determine the antimicrobial activity of each samples and the average of 3 replication was recorded.

Table 1: Antimicrobial activity of AgNPs synthesized from *Bacillus megaterium* (Avareage of three reading, Zone of inhibition of growth in mm)

Concentration (µl/ml)	*E. coli*	*S. aureus*	*P. aeureginosa*	*P. vulgaris*
30	17	20	17	14
60	20	22	19	17

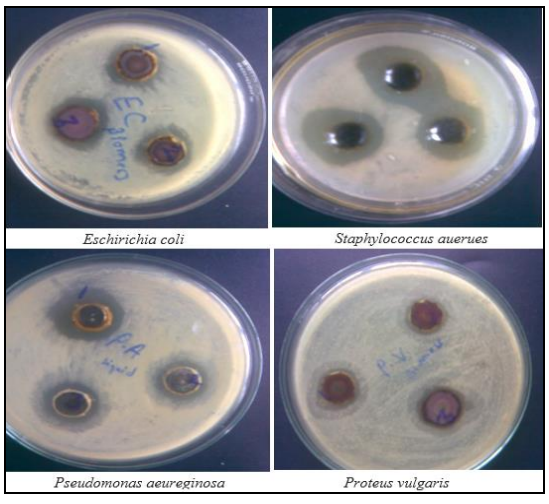

Fig 4: Antibacterial activity of silver nanoparticles by *Bacillus megaterium*

Conclusion

After conducting our study, we came to the following conclusions: the antimicrobial properties of biologically synthesized silver nanoparticles by *Bacillus megaterium* proved potent antimicrobial agent against *Staphylococcus aureus, Escherichia coli, Pseudomonasa aeruginosa* and *Proteus vulgaris.* This biosynthesized silver nanoparticles has many advantages over chemically derived nanoparticles and might an excellent means of developing and eco-friendly protocol

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