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Evaluation of antimicrobial activity of silver nanoparticles synthesized by green rout

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Abstract: Green rout for the synthesis of silver nanoparticles is an ideal process and is benign for environmental. Emergence of antimicrobial drug resistance forces the researcher to think about another alternative. Silver nanoparticle proved as an ideal antimicrobial for their control due to their physical and chemical properties. Thus in the present study, silver nanoparticles synthesized by using F4 bacterium isolated from the soil. Silver nanoparticle synthesis was achieved with in 72h. Synthesized nanoparticles were characterized by using UV-vis spectrophotometer, FTIR and SEM. Silver nanoparticle have challenging antimicrobial activity against *Pseudomonas aeruginosa, Escherichia coli and gram positive bacteria Bacillus subtilis* and *Staphylococcus aureus.*

Introduction:

Nanomaterial are playing very important role in many area due to their shape, size, physical, chemical, biological and optical properties. Nanoparticles of gold, silver and palladium are extensively applicable for biosensors synthesis[1,2], as antimicrobials[3], in drug delivery[4] and in gene therapy[5]. Silver nanoparticles have attracted attention of many researcher and medical practitioners due to their unique properties. Nanoparticles can be synthesized by chemical, physical and biological methods. Chemical and physical methods employed hazardous reducing agents for the synthesis of nanoparticles and products of these methods are usually perilous for the environment and biological systems. Therefore, green synthesis of nanoparticles is in great demands now days. Compared with physicochemical process, green route is free of hazardous product and cost effective[6, 7]. Consequently silver nanoparticle can be synthesized from microbial cells such as fungi and bacteria and plant extract[8]. Microorganisms are well known as nanofactory because they are reproduced very fast than any other organisms. The first report about synthesis of nanoparticles by bacteria was reported in 1984 by using *Pseudomonas stutzeri* AG259 isolated from silver mine[9,10,11]. Advantage of silver nanoparticles synthesized by bacteria is that they are under controlled size. Besides of these properties, silver nanoparticles are very good antimicrobial agents and using in medical field for the control of pathogens. As we know that emergence of multiple drug resistant microorganisms reported globally and are very difficult to control by antibiotic because they evolved resistance mechanism them. Hence, silver nanoparticle can be a good alternative of antibiotics. Silver ions have very good antimicrobial property and at nano level its antimicrobial properties enhance. Thus keeping these entire things in mind, the present study focused on the microbial synthesis of silver nanoparticles.

Hence, for the synthesis of silver nano particles, silver nitrate tolerant *Pseudomonas* sp. F4 isolated from soil. The silver nanoparticles was characterized its antimicrobial activity was checked against gram negative *P. aeruginosa, E. coli* as well as gram positive bacteria *B. subtilis* and *S. aureus*.

Materials and Methods:

Bacteria used in this study:

P. aeruginosa, E.coli and gram positive bacteria *B. subtilis* and *S. aureus* are clinical strains isolated from hospital waste. F4 bacteria was used for silver nanoparticles synthesis, it was isolated from the soil around the Panki Thermal Power Plant, Kanpur, India, all cultures were maintained on Luria Bertini Agar (LBA) or Luria Bertini Broth (LB) medium (Hi Media, India) at 37^{o}C

Synthesis of silver nanoparticles

For the synthesis of the silver nanoparticles, aqueous solution of silver nitrate was prepared in distilled water; reaction mixture contains the 90 ml silver nitrate (0.1mM) solution and 10 ml supernatant of 24 h log culture of isolated *Pseudomonas* sp. F4 as per modifications protocol of Kannan et al.[12]. The reaction mixture was incubated at 37^{o}C for the appearance of yellow to brown color and it was monitored at different time intervals (6, 12, 24, 48 and 72h) and formed nanoparticles were characterized for further study.

Characterization of silver nanoparticles

The preliminary characterization of the synthesized silver nanoparticles was carried out by UV-vis spectrophotometer (Thermo scientific). The spectra were recorded from 300-500 nm at resolution of 0.1 nm.

The shape and size of silver nanoparticles were determined by scanning electron microscope. Sample preparation for the electron microscopy was done by using 100 times diluted solution of silver nanoparticles, and then sample was allowed to dry for imaging. Dried sample use for scanning electron microscopy. The electron micrographs of the silver nanoparticles were taken by using Scanning Electron Microscope of JEOL (JSM 6490 LV).

Fourier transform infrared (FTIR) spectra were recorded at room temperature on a Thermo-Scientific (Nicole 6700) FTIR spectrometer. For the FTIR measurements of silver nanoparticles, a small amount of silver nanoparticles were taken for measurements.

Antimicrobial activity of Silver nanoparticle:

Antimicrobial activity of silver nanoparticles solution was checked against multiple drug resistant pathogens *E. coli, P. aeruginosa, B. subtilis* and *S. aureus by* well diffusion assay. Briefly, 100 µl log culture of *E. coli* with 1X10^{7}cfu/ml was spread on the Mueller Hinton Agar plate and kept for 10 min, after that wells were formed and 50 µl of silver nanoparticles solution was poured into the wells and incubated for 24h at 37^{o} C.

Results:

Silver nanoparticles have broad application in biomedical field, therefore present study focused on the synthesis of silver nanoparticles by using bacteria, which is ecofreindly and cost effective. Herein, F4 was isolated from the rhizosphere soil around the Panki Power plant, Kanpur. F4 is a gram positive bacterium and has potential to tolerate silver nitrate by 10mM. Hence, F4 was used for synthesis of silver nanoparticles. When reaction mixture of silver nitrate and supernatant of culture was incubated at 37^{o} C, it turned into brown color from milky white in 72h. Brown color of reaction mixture is indication of formation of silver nanoparticles (shown in figure-1).

At 0h 24h 72h

Figure-1) Synthesis of silver nanoparticles:

Characterization of silver nanoparticles:

The progress of reduction of silver nitrate by bacteria was evaluated by UV-vis spectrophotometer, which given the absorbance peak at 450nm (figure-2a). Primary characterization by UV-vis spectrophotometer revealed the presence of silver nanoparticles in the reaction mixture.

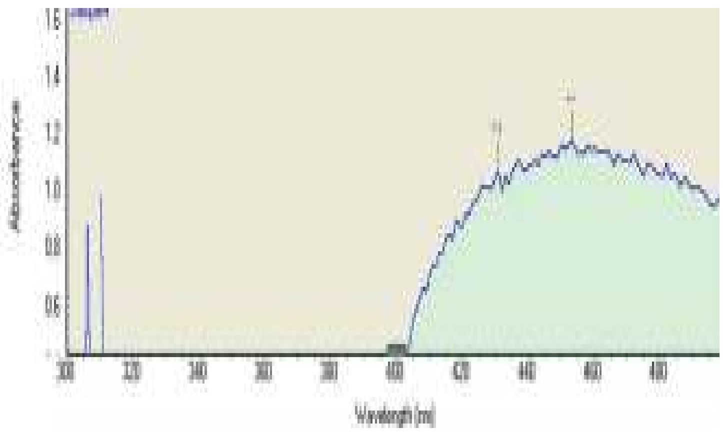
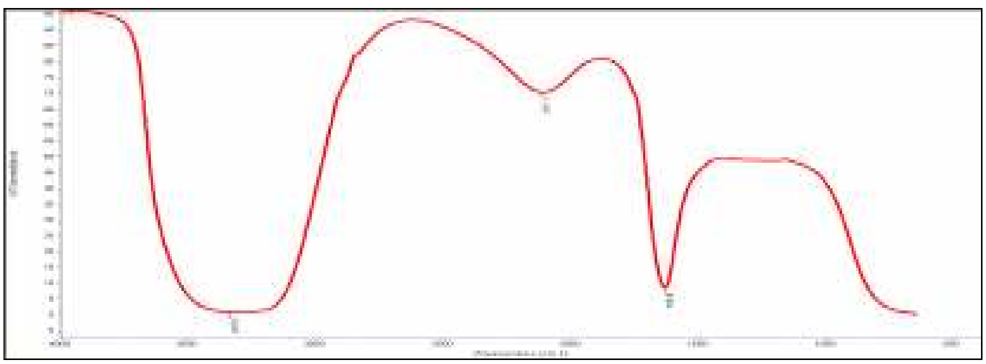


Figure-2) Characterization of silver nanoparticles: A. By UV-vis Spectrophotometer



Range	Functional groups
3500–3200 cm^{-1}	Alcohols, phenols or 1°, 2° amines, amides
2260–2100 cm^{-1}	Alkynes
1650–1580 cm^{-}	1° amines

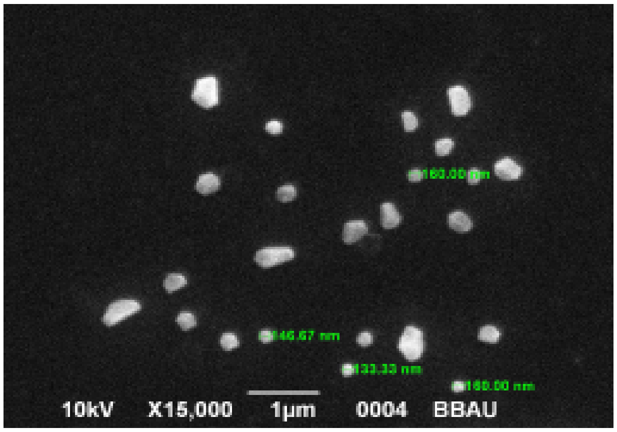
B. By Fourier transform infrared (FTIR) spectra

Fourier transform infra red (FTIR) spectroscopy was used to determined the presence of functional group in the silver nanoparticles solution. Figure 2b of FTIR spectra shows three peaks at 1635.9 cm^{-1}, 2117.1 cm^{-1} and 3331.5 cm^{-1} and these peaks correspond to primary amines, alkynes and phenols respectively. The amide stretches of 1635.9 cm^{-1} indicate the presence of proteins and these proteins can stabilize silver nanoparticles.

Morphological analysis of silver nanoparticles was done by SEM. SEM images revealed that the Silver nanoparticles are Hexagonal in shape and 133nm in diameter (figure-2c).

Antimicrobial activity of silver nanoparticles:

Antimicrobial activity of silver nanoparticles was evaluated against multiple drug resistant *P. aeruginosa, E. coli, B. subtilis* and *S. aureus.* Biosynthesized nanoparticles was given maximum inhibition zone against *P. aeruginosa* and *B. subtilis,* while for *E. coli* it showed very low inhibition activity (figure 3). Silver nanoparticles were unable to confer any inhibition activity against *S. aureus*.



C. Scanning Electron Microscopy of silver nanoparticles:

a) Pseudomonas aeruginosa b) Escherichia coli c) Bacillus subtilis d) Staphylococcus aureus
Figure-3) Antimicrobial activity of Silver nanoparticles:

Discussion:

Antimicrobial resistance is globally challenging problem for medical practitioners. As we know, there is no new antibiotic has been discovered since 1987. Therefore, the control of resistant pathogens has become very intricate by commercially available antibiotics. In the last few decades, nanoparticles have drawn the attention of medical researcher due to their extraordinary physical and chemical properties. Nanoparticles can be synthesized by physical, chemical and biological processes. But the physicochemically synthesized nanoparticles have rendered adverse effect on environment as well as on the biological system. However, the biologically synthesized nanoparticles are ecofreindly and cost effective[13]. Thus, the present study exploited the biological process for synthesis of silver nanoparticle by using F4 bacteria.

F4 bacteria isolated from the soil have potential to tolerate silver nitrate by 1mM, therefore F4 was further subjected for the synthesis of silver nanoparticles. Silver nanoparticles synthesis was achieved by bacteria with in 72h (figure-1). Initial characterization of silver nanoparticles was done by UV vis spectrophotometer[14]. Change in color from milky white to dark brown is due to surface plasmon resonance of exited silver nanoparticles[15,16]. Reduction of silver atom is actually two step reaction proposed by Sintubin et al.[17]. In the present study absorption peak of silver nanoparticles was get at 450nm (Figure-2). Absorbance indicates the particle size of reduced silver. In the present study, synthesis of silver nanoparticle was achieved by 0.1mM silver nitrate while Kalimuthu et al. [18] and Pandian et al.[19] reported synthesis of silver nanoparticles with 1mM.

The mechanism employed by bacteria for synthesis of silver nanoparticles is not still clear but the widely accepted mechanism is that the synthesis of silver nanoparticles achieved by the enzyme "Nitrate reductase" [20, 18]. The reduction mediated by the presence of the enzyme in the organism has been found to be responsible for the synthesis of silver nanoparticles. This mechanism was well described in *B. licheniformis* by Kalimuthu et al. [18].

Presence of functional group for the stabilization of silver nanoparticles was analyzed by FTIR. The FTIR data indicate the presence of three absorption spectra of 1635.9 cm^{-1}, 2117.1 cm^{-1} and 3331.5 cm^{-1}. The absorption peak of 3331.5 cm^{-1} correspond to OH stretch of carboxylic acid, while absorption peak of 2117.1 cm^{-1} correspond to CH stretching of aldehyde and 1635.9 cm^{-1} correspond to NH bending of primary amines of proteins that may be due to carboxylic stretch and amine deformation vibration [21]. Herein, Proteins function as capping group and stabilizes the nanoparticles [21], although the stability is produced by the binding of amino acid carbonyl group with the metals. Mallikarjuna[22] reported that biological molecules play dual role in synthesis and stabilizing of silver nanoparticles.

Morphological analysis of silver nanoparticles was determined by SEM. SEM micrograph showed that silver nanoparticles are roughly spherical shaped and are 133nm (figure-2c).

Antimicrobial activity of silver nanoparticles was evaluated against multiple drug resistant bacteria *P. aeruginosa, B. subtilis, E. coli* and *S. aureus*. In the present study, silver nanoparticle was given best antimicrobial activity against *P. aeruginosa* and *B. subtilis* (fig.3).

The mode of action of silver nanoparticle is not exactly clear but it is hypothesized that nanoparticles causes killing of microbial cell by binding to cell wall, DNA and promoting synthesis of free hydroxyl redicals [23, 24, 25]. It has also been reported that a strong reaction takes place between the silver ions and thiol groups of vital enzymes, which causes killing of cell [3].

Conclusion:

Silver nanoparticle synthesis was achieved by F4 bacterium. Microbial synthesized nanoparticles were stable at room temperature for several months. Silver nanoparticles have affinity to kill gram positive as well as gram negative cells. Thus, bacterial synthesized nanoparticle is stable ecofreindly cost effective.

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