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Characterization of Silver Nanoparticles Synthesizing Bacteria and Its Possible Use in Treatment of Multi Drug Resistant Isolate

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Abstract: Nanobiotechnology is a promising area to cater human life. Biological methods for the synthesis of silver nanoparticles are relatively cost effective process. It was aimed at synthesizing silver nanoparticles using water and soil borne bacterial isolates, characterization of silver nanoparticles using UV-Vis spectroscopy and Fourier transform infra red (FTIR) spectroscopy and its effect on multi drug resistant bacterial isolate. The biosynthesis of silver nanoparticles was evaluated in both bacterial biomass and culture supernatant of Bacillus, Pseudomonas and Escherichia coli in the presence of 1mM of AgNO3.On the basis of physical appearances of silver nano particles, Pseudomonas sp. was selected for synthesis. The absorbance spectra of reaction mixture of bacterial biomass and supernatant show the strong peak at 420 nm, indicating the presence of silver nanoparticles (AgNPs) using UV-Vis and FTIR spectrophotometry. The influence of synthesized AgNPs was tested against multi drug resistant (MDR) Staphylococcus sp. on Mueller Hinton agar. The multi antibiotics resistant Staphylococcus sp. showed antibiotic sensitivity against the antibiotic discs impregnated with silver nanoparticles. The characteristics of silver nanoparticles revealed its possible use in biomedical field.

Keywords: FTIR, MDR, Nanobiotechnology, *Pseudomonas* sp., Silver Nanoparticle

1. Introduction

Bionanotechnologyisan integration of biotechnology and nanotechnology. The idea of nanotechnology was coinedby Professor Richard Feynman [1]. Nanosilver has been used for more than 150 years in the form of colloidal silver, and registered as a biocidal agent in the United States [2]. Silver nitrate is often used as a precursor in the biosynthesis of silver nanoparticles. Silver nanoparticles (AgNPs) has being studied extensively in bionanotechnology mainly due totwomajor reasons, i) it isnon-toxicin nature, (ii) it is safe inorganic antibacterial agent and has ability of killing various kinds of disease causing microbes. Metallic nanoparticles exhibit several unusual optical, thermal, chemical and physical features [3]. Nanoparticles exist in several different morphologies such as spherical, cylindrical, platelets, tubes etc. [4].

Several physicochemical techniques are used to synthesize silver nanoparticles such as chemical reduction, electrochemical method, and photochemical reduction [5-7]. But, these methods have limitations like high operational cost and energy needs which make the synthesis cost competitive. Therefore, in view of these shortcomings of physicochemical methods, a cost-effective and energy efficient alternative for silver nanoparticles synthesis have been adapted using microorganisms [8].

Nowadays research is focused mainly on bacteria as a means of synthesizing nanoparticles due to their abundance, fast growth, easy to cultivate and their ability to adapt under extreme situations. The growth conditions such as temperature, presence of oxygen and incubation time are

being controlled for the synthesis of metallic nanoparticles [9]. Microbial synthesis of metal nanoparticles occurs either intracellularly or extracellularly. The intracellular synthesis requires biomass of culture. Whereas, when the culture supernatant is treated with aqueous solution of silver nitrate, then it forms silver nanoparticles extracellularly [10]. In the recent past, silver nanoparticles have gainedattention of researchers worldwide due to their potential antimicrobial activities [8, 11-13]. The properties of silver nanoparticles have made them applicable in severalareassuch as biomedical, drug delivery, water treatment, agricultures, etc.[14].

Keeping above in view, the present study was aimed at synthesizing silver nanoparticle using water and soil borne bacterial isolates, characterization of silver nanoparticles using UV-Vis and FTIR spectroscopy techniques and its effect on multi drug resistant bacterial isolates.

2. Materials and Methods

2.1. Sample Collection

The soil sample was collected fromSaket Dairy, Faizabad and waterfrom Ram kiPaidi, Saryu riverin Ayodhya. The collected water samples were stored in ice box and transported to laboratory for further processing.

2.2. Isolation, Morphological and Biochemical Characterization of Pseudomonas sp., Escherichia coli and Bacillus sp.

The bacterial cultures were isolatedon selective media*viz*., King's B agar medium (for *Pseudomonas*), Mac Conkey agar medium (for *E. coli*) and Pikovskays agar medium (for *Bacillus*). The samples were serially diluted following standard serial dilution method. Further, isolation of bacterial was done by inoculating 0.1 ml of diluted samples (10^{-4}, 10^{-5} and 10^{-6}) on selective media in petri dishes following spread plate method. The inoculated Petri dishes were incubated at different temperature (for *Bacillus* and *Pseudomonas* at ambient temperature, *E. coli* at 37°C). The isolated cultures were further identified and characterized in our laboratory using different morphological and biochemical tests as per Bergey's Manual of Determinative Bacteriology [15].

2.3. Screening of Bacterial Isolates for Silver Nanoparticles Synthesis

The selected bacterial cultures were inoculated in 500 ml flask containing 250 ml nutrient broth or selected media for the screening of silver nanoparticles synthesizing bacteria,. The flasks were incubated in a rotating shaker set at 100 rpm for48 h atambient temperature. The culture was centrifuged at 12000 rpm for 10 minutes. The biomass and supernatant were separated and used separately for the synthesis of silver nanoparticles. The supernatant was used for extracellular production and biomass for intracellular production of nanoparticles. The modified method of Shivakrishna et al. [16] was used for nanoparticle synthesis. Two grams of biomass was added with 1mM of silver nitrate for the intracellular synthesis, the control set of experiment consisted heat killed biomass added with silver nitrate. The extracellular synthesis of silver nanoparticles was carried out by adding 1 mM of silver nitrate to 99 ml of supernatant, the control set containedonly silver nitrate and 99 ml of distilled water and another set of control for supernatant were used without addition of silver nitrate. The biosynthesis of silver nanoparticles using both biomass and supernatant were separately investigated primarily through the observation of colour change of the experimental sample in the presence of 1mM of silver nitrate.

2.4. UV-Visible Spectral and FTIR Analyses

The synthesized silver nanoparticle was the characterized by UV visible spectrophotometer. FTIR spectroscopy measurements, the biotransformed products present in extracellular filtrate were freeze-dried by lyophilization process and diluted with potassium bromide. The palette of KBr and AgNPs was made using KBr die set and applying hydraulic pressure of 12 tons. The spectrum was recorded on a FT-IR instrument (Thermo Scientific) with diffuse reflectance mode (DRS-800) attachment. All measurements were carried out in the range of 200–7000 cm^{-1}wavenumbers [17].

2.5. Effect of Synthesized Nanoparticles on Multi Drug Resistant Bacteria

The filter sterilized silver nanoparticles were used and impregnated with antibiotic discs. The *Staphylococcus* culture was spread on Mueller Hinton agar medium (MHA). Treated antibiotic discs were placed on the seeded Mueller Hinton agar medium, and the petri dishes were incubated at 37°C for 24 h. The control set of experiment was prepared by same method excluding the antibiotic not treated with SNPs. Following 24 h, the zone of inhibition was measured.

3. Results and Discussion

3.1. Isolation and Biochemical Identification of Nanoparticles Synthesizing Bacteria

Three different bacterial cultures were isolated on their selective medium after24h of incubation. The colony forming unit (cfu) values of nanoparticles synthesizing bacteriasuch as *Bacillus, Pseudomonas* and *Escherichia coli* on different media was calculated as 153, 147 and 132, respectively in dilution 10^{-4},10^{-5} and 10^{-6}. Further bacterial colonies were identified on the basis of their morphological and biochemicalfeatures. The results of morphological and biochemical tests revealed the identity of our efficient isolates as *Bacillus, Pseudomonas* and *E. coli* as per Bergey's Manual of Determinative Bacteriology (Table 1).

Table 1. *Morphological and biochemical Characterization of selected Bacterial cultures.*

Morphological Test	**Bacterial Isolates**		
	***Bacillus* sp.**	***Pseudomonas* sp.**	***E. coli***
Colonies appearance	Convex, smooth, transparent	Colourless, convex, smooth, fluorescent, circumference	Transparent colonies, smooth margin, convex
Negative staining	Small rod, arranged inchain	Short rod	Cocci, diplococci, rod,
Gram staining	Gram-positive	Gram-negative	Gram-negative
BIOCHEMICAL TEST			
Oxidase	Negative	Positive	Positive
Methylred (MR)	Negative	Negative	Positive
VogesProskaur (VP)	Positive	Negative	Negative
Oxidative Fermentation (OF)	Positive	Positive	Positive
Indole	Negative	Negative	Positive
Citrate	Positive	Positive	Negative
Urease		Negative	Negative
Nitrate Reduction (NR)	Positive	Positive	Positive
Hydrogen Sulphide production		Negative	Negative
Gas	Negative	Positive	Positive
Catalase	Positive	Positive	Positive
CARBOHYDRATE UTILIZATION TEST			
Glucose	Positive	Negative	Positive
Fructose	Positive	Negative	Negative
Maltose	Positive	Negative	Negative
Lactose	Variable	Negative	Positive
Sucrose	Positive	Sucrose	Variable
Arabinose	Positive	Negative	Positive
Mannitol	Positive	Positive	Negative

3.2. Biosynthesis of Silver Nanoparticles

The biosynthesis of silver nanoparticles were investigated using both biomass and supernatant separately primarily through the observation of colour change of the experimental sample in the presence of 1mM of $AgNO_3$. A colour change from pale yellow to brown occurred in both bacterial biomass and supernatant within 24 h. The positive result as observed by the formation brown colour was maintained throughout the 72 h (not shown). At the same time, experimental control containing heat killed biomass or supernatant without silver nitrate showed no colour change (not shown). The visual change of colour from yellow to reddish brown might be due to excitation of surface plasmon resonance in silver nanoparticle s[16, 18]. Even though the colour change was observed for sample containing, both biomass and supernatant, however further experiments were continued with extracellular samples due to comparative advantage of extracellular better synthesis over intracellular fraction.

3.3. Characterization of Synthesized Silver Nanoparticles

The synthesized silver nanoparticles were then characterized by UV spectrophotometer**.** The UV-visible spectra recorded at different time intervals showed increased absorbance with increasing time of incubation. The absorbance spectra of reaction mixture containing aqueous solution of 1mM silver nitrate and the pellet ofselected bacterial isolatesafter incubation. The bands corresponding to the surface plasmon resonance were observed between 410 to 430 nm employing spectrophotometrically [19]. However, the peak was observed at 420 nmwas evident of the presence of silver nanoparticlesin both bacterial culture supernatant and biomass (Figures 1 a, b). Similar to our findings, other researchers have also reported the formation of silver nanopartícles by exhibiting the typical surface plasmon absorption maxima at nearly 420 nm from the UV–Vis spectrum [16, 18, 19].

The characterization of the silver nanoparticles was also investigated by analysing FTIR spectra. The spectral analyses were done for control (Figure 2a) and experimental samples (Figure 2b) using FTIR spectroscopy. The absorption spectra observed also showed the presence of the silver nanoparticles in bacterial culture supernatant (Figure 2b). The mechanism for silver nanoparticles biosynthesis involves nitrate reductase enzyme [20]. The bands observed between 1000 cm-1 to 1600 cm-1 might be due to stretching vibrations of C= O, C-N and O-H functional groups, respectively. Other researchers also reported similar findings. Mohanta et al. [13] also reported that the C= O and C-N stretching which are usually found in the proteins involve in the reduction of the metal ions. The findings revealed that the above functional groups might play role in silver nanoparticles synthesis.

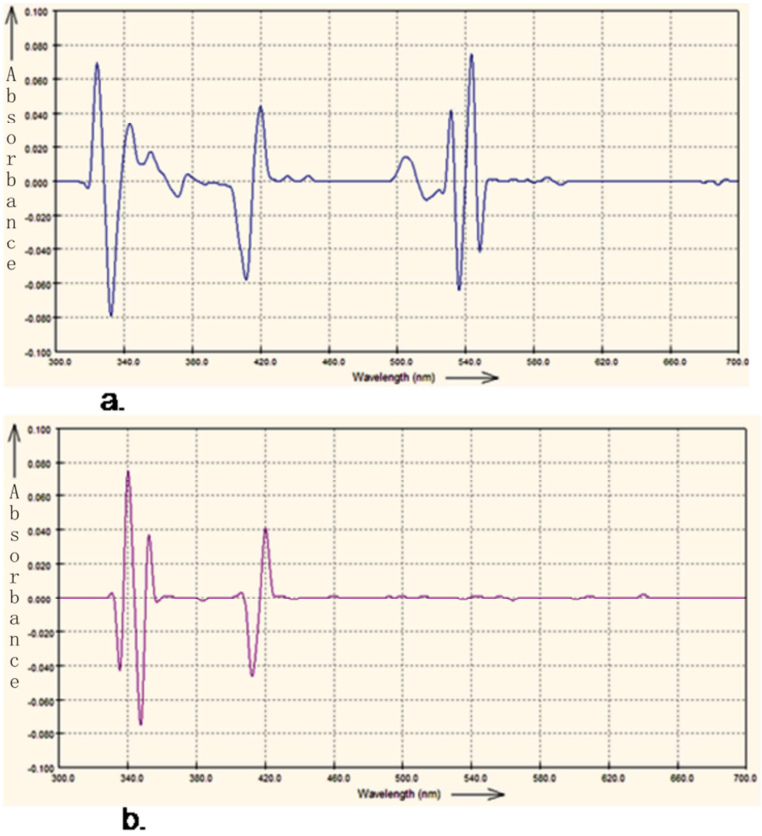

Figure 1. *UV–Vis absorption spectra of Pseudomonas sp. (a) biomass and (b) culture supernatant after 24 h incubation (showing the presence of silver nanoparticles).*

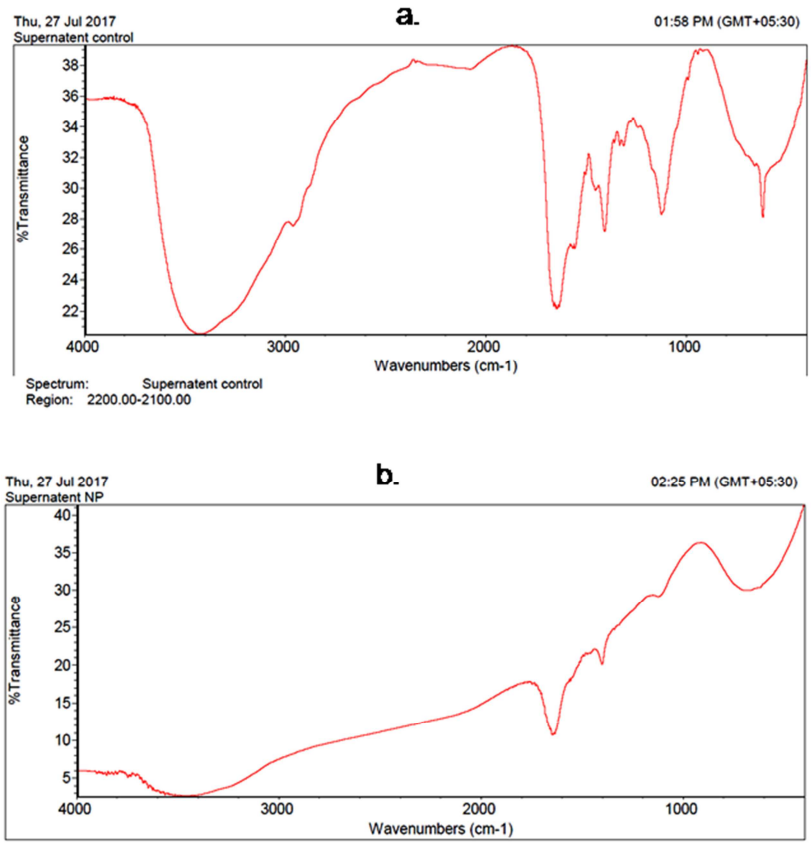

Figure 2. *FTIR spectra recorded with synthesized silver nanoparticles in bacterial supernatant, (a) experimental and (b) control sample.*

3.4. Antibacterial Effect of Silver Nanoparticles

Silver and its derivatives are widely used in medicine for a long time in the treatment of bacterial infections. Discs diffusion test was performed for *Staphylococcus* sp. using different antibiotics. The bacterium was resistant to all tested antibiotics. However, *Staphylococcus* sp. was found to be sensitive for the most of tested antibiotic (doxycyclin, ciprofloxacin and ceftazidime) when silver nanoparticles impregnated antibiotic discs were used compared to nonimpregnated discsonMueller Hinton Agar medium (Table 2). Other researcher also investigated the antibacterial property of silver nanoparticles against multidrug resistant organisms [21]. The mechanism of the bactericidal effect of silver nanoparticles is not well-known. Whereas silver nanoparticles may attach to the surface of cell membrane, and disturb its physiological functions [18]. However, many researchers proposed the possible mechanisms of antibacterial effect of silver nanoparticles such as inactivation of main cellular proteins, impairment of genetic materials and enzyme degradation by silver ions [22, 23].

Table 2. *Antimicrobial activity of silver nanoparticles against multiple antibiotic resistant Staphylococcus sp.*

Antibiotics	Results	
	Antibiotic disc	Silver nanoparticles impregnated antibiotic disc
Doxycyclin (DO)	Resistant	Sensitive
Ciprofloxacin (CIP)	Resistant	Sensitive
Ceftazidime (CAC)	Resistant	Sensitive

4. Conclusions

The present study emphasizes the use of bacteria for silver nanoparticles synthesis with potent biological effect such as characterization of silver nanoparticles having antibacterial activity. The silver nanoparticles were synthesized in both bacterial biomass and culture in the presence of 1mM of $AgNO_3$. The characterization of silver nanoparticles synthesized by *Pseudomonas* sp. was observed by absorbance spectra using UV-Vis and FTIR spectrophotometry. The synthesized nanoparticles were made sensitive to antibiotics for which the isolate *Staphylococcus* sp. was previously resistant before amendment of silver nanoparticles. The study suggested the possible use of microbially synthesized silver nanoparticles against antibiotic resistant bacteria which is a major public health concern.

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