

# Rapid synthesis of silver nanoparticles by *Pseudomonas stutzeri* isolated from textile soil under optimised conditions and evaluation of their antimicrobial and cytotoxicity properties

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**Abstract:** Present study utilised textile soil isolated bacterium *Pseudomonas stutzeri* to synthesise extracellular silver nanoparticles (AgNPs) under optimised conditions. The synthesised AgNPs were characterised using ultraviolet-visible spectroscopy, Fourier transform infrared spectroscopy (FTIR) and transmission electron microscopy (TEM). Optimisation showed AgNPs synthesis within 8 h using 2mM Ag nitrate at pH9, temperature 80°C and maximum absorbance toward 400 nm. TEM analysis revealed spherical shape AgNPs and reduction in size upto 8 nm was observed under optimised conditions. FTIR spectra confirmed presence of proteins bound to AgNPs act as reducing agent. AgNPs showed strong antibacterial activity against multi-drug resistant (MDR) *Escherichia coli* and *Klebsiella pneumoniae* as demonstrated by disc diffusion and colony forming unit assays. Zone of inhibition increased with increasing concentration of AgNPs with maximum of 19 mm against *E. coli* and 17 mm against *K. pneumoniae* at concentration of 2 µg/disc. Furthermore, AgNPs did not show any cytotoxic effects on human epithelial cells as demonstrated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay even at 2 µg/ml concentration of AgNPs. The results of the present study suggest that AgNPs can be synthesised rapidly under optimised conditions and show strong antimicrobial property against MDR pathogens without having toxicity effect on human epithelial cells.

## 1 Introduction

Nanotechnology, deals with the synthesis and applications of nanoscale materials in diverse interdisciplinary fields such as physics, chemistry, biology, medicine and agriculture [1]. Numerous metals such as silver (Ag) [2], gold [3], copper [4], zinc [5], iron [6], palladium [7], selenium [8] and titanium [9] have been investigated at the nanoscale. Among all the metallic nanoparticles Ag nanoparticles (AgNPs) have received greatest attention because of strong antimicrobial action [10] and hence used in nanomedicine.

Vast applications and rapid utilisation of engineered nanoparticles would inevitably lead to the release of these materials into the environment and cause toxicity to different organisms. Various techniques such as physical and biological means have been utilised for the synthesis of AgNPs. However, biological route of synthesis using bacteria, yeast, fungi, algae and plants is gaining attention due to cost-effectiveness, least toxicity and better optimisation control [11].

Microorganisms utilise both extracellular and intracellular routes for the synthesis of nanoparticles [12]. Extracellular biosynthesis is preferable as it reduces the complex downstream processing than the intracellular biosynthesis which requires additional steps such as cell lysis by ultra-sonicator or suitable detergent treatment for purification of synthesised nanoparticles [13]. A number of bacterial species including *Escherichia coli* [14], *Ureibacillus thermosphaericus* [15], *Staphylococcus aureus* [16], *Bacillus thuringiensis* [17], *Pseudomonas aeruginosa* [18] and *Bacillus cereus* [19] have been explored to synthesise AgNPs. The limitation behind bacteriogenic synthesis of nanoparticle is slow rate of synthesis, non-uniform size of nanoparticles and polydispersity [20]. Moreover, optimisation studies of various parameters such as Ag nitrate (AgNO<sub>3</sub>) concentration, pH and temperature for enhanced and rapid synthesis of AgNPs are lacking.

The growing resistance of pathogenic bacterial strains to traditional antibacterial treatments has encouraged the development of alternate strategies to control infections. The development of non-toxic methods of synthesising nanoparticles would be a major

step in nanotechnology to allow their application in medicine. Being strong antibacterial, AgNPs because of its small size with increased surface have been used as biocide in different formulations as well as in increasing number of consumer and medical products [21]. AgNPs were reported to have antibacterial activity against various pathogenic bacteria both Gram positive and Gram negative [22]. Several mechanisms including penetration of bacterial cell wall, damaging the cell membrane, generation of free radicals, inhibition of signal transduction pathways and DNA damage have been reported [23, 24].

Nanomaterials synthesised have shown some profound effect on human health. Nanoparticles have ability to enter human body in various ways and able to cause toxicity mainly by generation of reactive oxygen species and genotoxicity [25]. Many nanoparticles have also been reported to induce inflammatory response through activation of expression of tumour necrosis factor alpha. Nanoparticles can also lead to an increased expression of other pro-inflammatory cytokines such as interleukins 2, 6, 8 and 10 (IL-2, IL-6, IL-8 and IL-10) [26, 27].

The present study deals with the extracellular biosynthesis of AgNPs, using cell filtrate of *P. stutzeri* isolated from soil. Furthermore, characterisation and optimisation for rapid AgNPs synthesis were carried out. Antimicrobial and toxicity studies have also been elucidated.

## 2 Materials and methods

### 2.1 Chemical, culture media and bacterial strains

The chemical and culture media used in the study were purchased from Sisco Research Laboratories (SRL), India and Himedia, India, respectively. The test organisms including *E. coli* and *Klebsiella pneumoniae* were procured from All India Institute of Medical Science (AIIMS), New Delhi.

## 2.2 Sample collection and isolation of bacteria

The soil sample was collected from textile area near Sanganer, Jaipur, Rajasthan, India at 10 cm depth in sterilised zip-lock bag. The collected soil sample was stored in ice box and then transported to the laboratory within 6 h. About 1 g of soil was serially diluted and spread over nutrient agar followed by incubation at 37°C for overnight. Ten colonies were picked randomly and sub-cultured to obtain pure culture.

## 2.3 Screening for extracellular synthesis of AgNPs

The ten isolates were freshly inoculated in 100 ml nutrient broth in Erlenmeyer flask and incubated in orbital shaker for 24 h at 37°C and at 200 rpm. Culture filtrates were obtained by centrifugation at 10,000 rpm for 10 min. The supernatant was challenged with 2.0 mM AgNO<sub>3</sub> solution and incubated in dark condition at 37°C. The isolate showing maximum AgNPs synthesis was characterised by morphological and biochemical tests as per the procedures outlined in Bergey's Manual of Determinative Bacteriology [28]. The bacterium was also characterised based on 16S rDNA technique at Xcleries genomics laboratory. The bacterium was identified as *P. stutzeri*.

## 2.4 Optimisation studies for rapid AgNPs synthesis

Different reaction parameters such as AgNO<sub>3</sub> concentration, pH and temperature were optimised to obtain maximum production of AgNPs [29].

**2.4.1 Effect of AgNO<sub>3</sub> concentration:** Nanoparticles synthesis is dependent on substrate concentration. AgNPs were synthesised using different concentrations of AgNO<sub>3</sub> from 0.2 to 2.5 mM. The sample was analysed with ultraviolet-visible (UV-vis) absorption spectroscopy at regular time interval from 24 to 144 h.

**2.4.2 Effect of pH:** pH plays a very important role in growth and enzyme production which is required for the biosynthesis of AgNPs. Different pH values ranging from 3.0 to 11.0 were used to study the influence of pH on AgNPs production and UV-vis absorption spectra were recorded from 24 to 144 h after addition of AgNO<sub>3</sub>.

**2.4.3 Effect of temperature:** Optimisation studies with respect to temperature were carried out with temperature ranging from 20 to 80°C for AgNPs production. UV-vis absorption spectra were taken at different time interval from 0 to 24 h.

## 2.5 Characterisation of AgNPs

**2.5.1 UV-vis spectroscopy analysis:** AgNO<sub>3</sub> treated culture filtrate of *P. stutzeri* was monitored for reduction of Ag ions by reading the sample aliquots (1 ml) at different time intervals on UV-vis spectrophotometer (Thermo scientific/Biomate 3S) at a resolution of 1 nm.

**2.5.2 FTIR spectroscopy analysis:** The interaction between protein and AgNPs was analysed by Fourier transform infrared (FTIR) spectroscopy. The bio-transformed products present in culture supernatant were freeze-dried and FTIR spectrum was recorded on FTIR (Shimadzu, India) in the range of 400–4000 cm<sup>-1</sup> at a resolution of 4 cm<sup>-1</sup>.

**2.5.3 Transmission electron microscopy (TEM) analysis:** Morphological characterisation including shape and size of AgNPs synthesised by *P. stutzeri* culture supernatant was done by taking a drop of solution containing AgNPs loaded on copper grids in transmission electron microscope (Jeol, USA). TEM micrographs were taken and size and shapes were analysed.

## 2.6 Determination of antibacterial activity

### 2.6.1 Antibacterial activity by disc diffusion assay:

Antibacterial activity of AgNPs synthesised from *P. stutzeri* was performed by disc diffusion method against *E. coli* and *K. pneumoniae*. In brief, overnight grown bacterial cultures were spread onto Muller-Hinton agar followed by 10 µl of AgNPs (from 50, 100 and 200 µg/ml concentration of AgNPs) soaked sterile paper discs were kept on nutrient agar so as to achieve different 0.5, 1 and 2 µg of AgNPs/disc. The plates were kept overnight at 37°C and zone of inhibition was measured. Ampicillin (30 µg/disc) was used to show *E. coli* and *K. pneumoniae* as multi-drug resistant (MDR) strains [30].

**2.6.2 Antibacterial activity in suspension:** About 10<sup>5</sup> colony forming units (CFUs) per millilitre of *E. coli* and *K. pneumoniae* in phosphate buffer saline (PBS) and different concentrations of AgNPs (0.5, 1, 1.5 and 2 µg/ml) were mixed and incubated at 37°C for 1 h. The samples were then plated on nutrient agar and incubated at 37°C. The numbers of the viable cells were determined by colony counting and 1 ml of pure PBS was used as a negative control. Bacterial killing percentage (% kill) was defined as  $(N_{\text{PBS}} - N_{\text{sample}}) / N_{\text{PBS}} \times 100$ , where  $N_{\text{PBS}}$  and  $N_{\text{sample}}$  represent the number of bacteria in PBS and the sample, respectively [31].

### 2.7 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) viability assay

Cell viability was assessed by MTT assays. For MTT assay,  $2 \times 10^3$  cells/well were seeded into sterile 96-well plate for 24 h and then exposed to increasing concentrations of AgNPs (0.5, 1, 1.5 and 2 µg/ml). After 24 h of exposure, cells were washed with PBS and 0.5 µg/µl MTT (Sigma) was added and cells incubated for additional 4 h at 37°C in the dark. Medium was removed and formazan crystals were dissolved in Dimethyl Sulphoxide (DMSO) and cellular metabolism was determined by monitoring the colour development at 570 nm, in a multi-well scanning spectrophotometer. Cells without AgNPs exposure were used as control. Cells viability percentage (%) was calculated as  $(\text{sample Abs}_{570-655 \text{ nm}}) / (\text{control Abs}_{570-655 \text{ nm}}) \times 100$  [32].

### 2.8 Hemolysis assay

Hemolytic activity of AgNPs was evaluated by measuring the hemoglobin release from the erythrocytes [red blood cell (RBC)] on interaction with nanoparticles under *in vitro* conditions. About 5 ml blood was collected from human volunteer in a tube containing ethylenediaminetetraacetic acid and was centrifuged at 2000 rpm for 10 min. The supernatant and buffy coat was discarded and the pellet containing RBCs were washed with PBS. About 10<sup>8</sup> cells of RBCs diluted in PBS were added to different concentrations of AgNPs (50, 100 and 200 µg/ml) and incubated at 37°C with mild shaking for 1 h. Then, the tubes were centrifuged at 2000 rpm for 10 min and the supernatant were read at 540 nm. RBCs in 1% triton X-100 were taken for complete lysis and considered as positive control. Percentage hemolysis (%) was calculated as  $(\text{Abs}_{540 \text{ nm}} \text{ in the AgNP solution} - \text{Abs}_{540 \text{ nm}} \text{ in PBS}) / (\text{Abs}_{540 \text{ nm}} \text{ in 1\% Triton X-100} - \text{Abs}_{540 \text{ nm}} \text{ in PBS}) \times 100$  [32].

## 3 Results and discussion

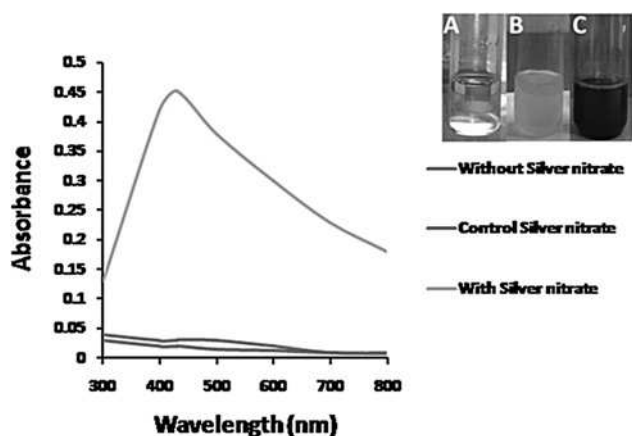
### 3.1 Molecular identification of bacterial isolate

Bacteria were isolated from textile soil samples of Jaipur, Rajasthan, India by serial dilution and sub-culturing. Randomly, ten bacterial isolates were selected and screened for AgNPs synthesis. Strain showing AgNP formation by UV-vis spectrophotometer was characterised. Morphological and biochemical characteristics of isolate have been outlined in Table 1. The partially amplified (803 bp) and sequenced 16S rRNA gene was uploaded to the National Center for Biotechnology Information website to search for similarity

**Table 1** Morphological and biochemical characteristics of *Pseudomonas stutzeri*

Characters	<i>P. stutzeri</i>
morphology	
shape	rod
gram stain	–
endospore	–
IMVIC	–
indole	–
methyl red	–
Voges–Proskauer	+
citrate	+

IMVIC, Indole Methyl red Voges-Proskauer and Citrate test



**Fig. 1** UV-Vis spectra of AgNPs synthesized using culture supernatant of *P. stutzeri* at 2mM AgNO<sub>3</sub>, pH 7 and temperature 30°C.

- a Blank AgNO<sub>3</sub> solution
- b Blank culture supernatant
- c Dark color AgNPs solution

to known DNA sequences and to confirm the species of the isolate. The nucleotide sequences coding for the 16S rRNA gene after Basic Local Alignment Search Tool query revealed that this gene is 99% homologous to *P. stutzeri* (BPRIST027). The nucleotide sequences coding for the 16S rRNA gene of *P. stutzeri* have been submitted to the GenBank database under accession numbers KU530223.

### 3.2 Extracellular synthesis of AgNPs

Visual observation of AgNO<sub>3</sub> showed a change in colour intensity from light (Fig. 1B inset) to dark (Fig. 1C inset). No change was observed in culture supernatant without AgNO<sub>3</sub> (Fig. 1B inset). The appearance of a dark colour (Fig. 1C inset) in AgNO<sub>3</sub> treated culture supernatant suggested the formation of AgNPs. The oscillation waves of electrons of AgNPs are in resonance with the

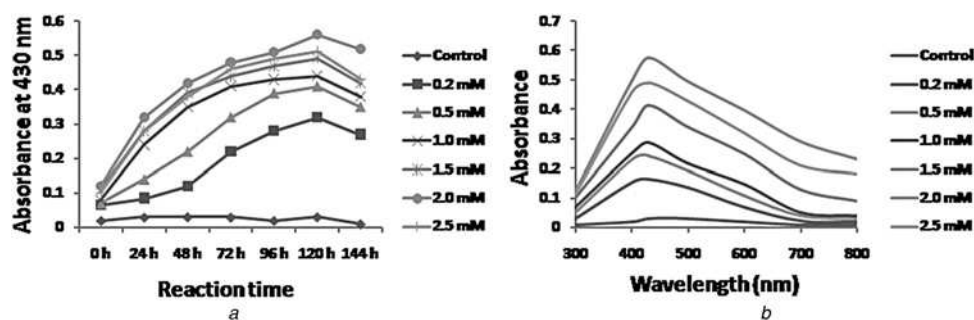
light waves gives rise to a unique surface plasmon resonance (SPR) absorption band (400–440 nm), which is also the origin of the observed colour [33]. The extracellular synthesis of AgNPs using *P. stutzeri* was monitored in UV–vis spectroscopy. Graph shown a strong peak at 430 nm (Fig. 1), indicated an SPR, having nanoparticles with sizes ranging from 2 to 100 nm. Similar results were reported by Singh [30], who also synthesised AgNPs from *Acinetobacter calcoaceticus*. The precise mechanism involved in bacteria mediated synthesis of AgNPs has not been known. However, it can be supposed that nitrate reductase secreted by microbes help in the bioreduction of metal ions to metal nanoparticles [34]. Furthermore, Kalimuthu *et al.* [35] confirmed the *Bacillus licheniformis* mediated secretion of nitrate reductase responsible for the reduction of Ag<sup>+</sup> to nanoparticles.

### 3.3 Optimisation studies for AgNPs production

**3.3.1 Effect of AgNO<sub>3</sub> concentration:** Different concentrations of AgNO<sub>3</sub> solution (0.2–2.5 mM) was used to synthesise AgNPs using *P. stutzeri* culture supernatant and monitored at regular interval from 24 to 144 h. At 0.2mM AgNO<sub>3</sub> concentration synthesis of AgNPs is very low which increases up to 2 mM AgNO<sub>3</sub> at 120 h of incubation (Fig. 2a). Amount of AgNPs decreased after 144 h of incubation indicating complete reduction of Ag<sup>+</sup> at 120 h. Maximum peak at 2 mM concentration is about 430 nm (Fig. 2b) and further increase in AgNO<sub>3</sub> concentration led to decrease in the synthesis of AgNPs. This can be explained on the basis of enzyme–substrate kinetics where active site of enzyme catalysing reduction is saturated with the Ag ions and no more site is available for ions to be reduced; therefore, no further increase in AgNPs synthesis at higher concentration of AgNO<sub>3</sub> [36].

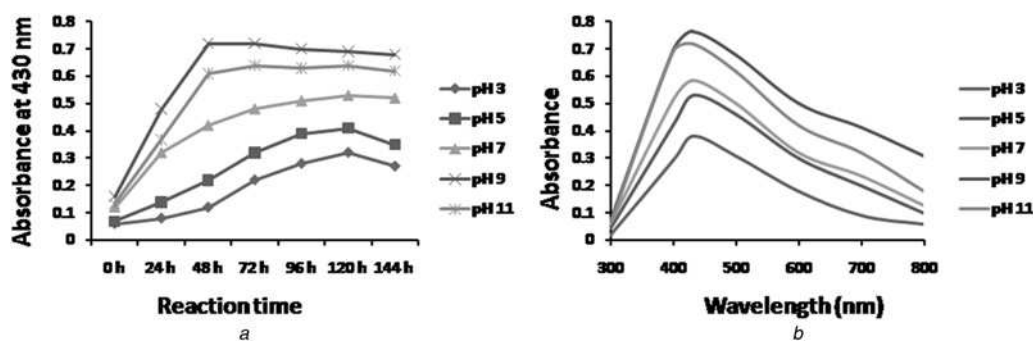
**3.3.2 Effect of pH:** To optimise the pH for maximum AgNPs synthesis, AgNO<sub>3</sub> was added in the culture supernatant having different pH (3, 5, 7, 9 and 11). AgNPs were found to be synthesised within 48 h at pH 9 and remain stable up to 144 h (Fig. 3a). UV–vis spectra demonstrated maximum absorbance peak of 430 nm (Fig. 3b). It may be due to increased availability of OH<sup>–</sup> at pH 9, which helps in complete reduction of Ag<sup>+</sup> into AgNPs by providing electrons [36]. However, increase in pH up to 11 and decreased synthesis of AgNPs were observed due to inactivation of enzyme responsible for AgNPs synthesis. Our data are in agreement with Sintubin *et al.* [37], who also reported enhanced AgNPs production at alkaline pH using lactic acid bacteria due to more competition between H<sup>+</sup> and Ag<sup>+</sup> for negatively charged OH<sup>–</sup>. On the contrary, acidic pH led to increased H<sup>+</sup> concentration which may denature enzymes responsible for AgNPs synthesis [38].

**3.3.3 Effect of temperature:** Temperature is an important factor affecting AgNPs production. Effect of temperatures on AgNPs production by *P. stutzeri* was carried out at different temperatures from 20 to 80°C with a difference of 20°C, 2 mM AgNO<sub>3</sub>, pH 9 and monitored at regular time interval. At 80°C, synthesis of



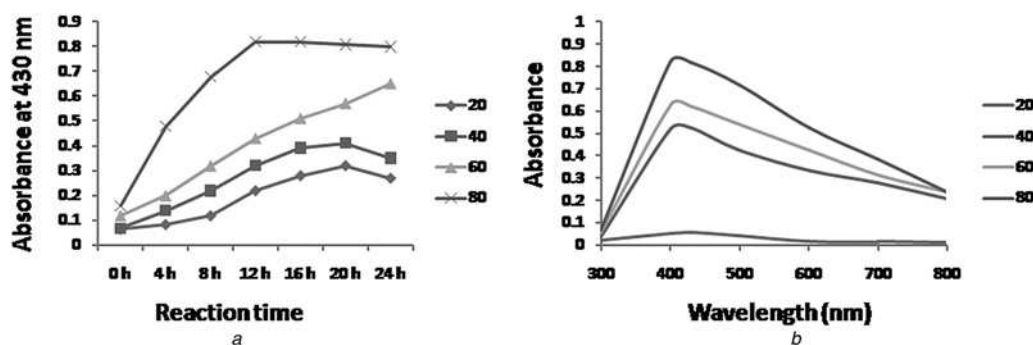
**Fig. 2** Optimisation of AgNO<sub>3</sub> concentration for AgNP synthesis

- a Time-dependent AgNP synthesis at 30°C using different concentrations of AgNO<sub>3</sub>
- b UV–vis spectra of AgNP synthesis at 30°C after 120 h obtained with different concentrations of AgNO<sub>3</sub>



**Fig. 3** Optimisation of pH for AgNP synthesis

*a* Time-dependent AgNP synthesis at 30°C using 2 mM concentrations of AgNO<sub>3</sub> at different pH  
*b* UV-vis spectra of AgNPs synthesis at 30°C after 120 h at different pH



**Fig. 4** Optimisation of temperature for AgNP synthesis

*a* Time-dependent AgNP synthesis at different temperatures using 2 mM concentrations of AgNO<sub>3</sub> at pH 9  
*b* UV-vis spectra of AgNPs synthesis at different temperatures after 120 h at pH 9

AgNPs was observed within 8–10 h and remains stable for longer time period indicated rapid and stabilised synthesis (Fig. 4*a*). There was little shift in peak from 430 nm toward 400 nm as temperature increases from 40 to 80°C (Fig. 4*b*). This may be due to increase in kinetic energy of the AgNPs in the solution at high temperature, which leads to faster synthesis rate [39]. Similar results have been found by Gurunathan *et al.* [40], who used *E. coli* for AgNPs synthesis and found maximum synthesis at 5 mM AgNO<sub>3</sub>, 60°C temperature and pH 10.

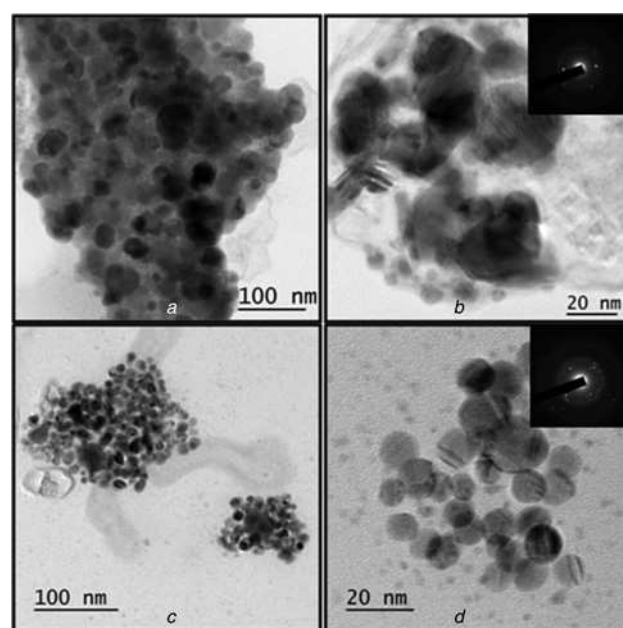
### 3.4 TEM characterisation of synthesised AgNPs

To determine the shape, size and morphology of *P. stutzeri* mediated synthesised AgNPs under optimised conditions, TEM measurements were carried out. TEM micrograph revealed that the nanoparticles are spherical in shape and well dispersed without agglomeration. Selected area electron diffraction (SAED) pattern revealed the crystalline nature of AgNPs synthesised using culture supernatant of *P. stutzeri* (Fig. 5*b* inset and 5*d* inset). The average particle size of AgNPs synthesised by *P. stutzeri* ranges from 15 to 20 nm (Figs. 5A and B). However, under optimised conditions, smaller size of AgNPs was synthesised with average size of 8 nm (Figs. 5C and D). This reduction of AgNPs size will result in increased surface area of contact with microorganisms and make it better bactericidal [41]. In another study, AgNPs of average size 27.5 nm were synthesised using culture filtrate of *P. aeruginosa* [18]. Furthermore, the presence of capping material around each particle as revealed by well separated AgNPs will improve stability and increase chances of attachment on bacterial surface [42].

### 3.5 FTIR characterisation of synthesised AgNPs

FTIR measurement of the dried and powdered sample was carried out to identify the presence of proteins surrounding AgNPs that

could be responsible for synthesis and stabilisation of AgNPs. FTIR spectrum revealed the presence of peak around 1630 cm<sup>-1</sup> which is assigned as absorption peak of –C=O and –C=C– stretching, and stretch vibration of –C=C– and is assigned to the



**Fig. 5** Morphology of AgNPs synthesised under normal and optimised conditions. TEM images for AgNPs synthesised at 30°C, pH 7 with 2 mM AgNO<sub>3</sub> at low (A) and high magnification (B). TEM images for AgNPs synthesised at 80°C, pH 9 with 2 mM AgNO<sub>3</sub> at low (C) and high magnification (D). Inset shows SAED pattern of AgNPs

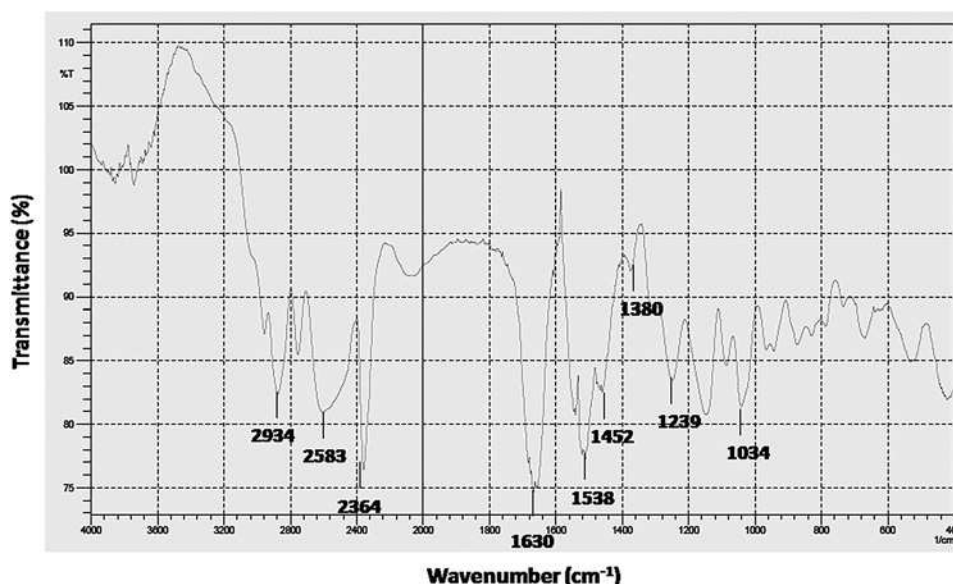


Fig. 6 FTIR spectra of synthesised AgNPs

amide I bonds of proteins (Fig. 6). The presence of peak at  $1452\text{ cm}^{-1}$  may be assigned to symmetric stretching vibrations of  $-\text{COO}^-$  groups of amino acid residues with free carboxylate groups in the protein. The peaks seen at  $1380$  and  $1034\text{ cm}^{-1}$  correspond to  $-\text{C}-\text{N}$  stretching vibrations. The presence of band at  $1538$  and  $1239\text{ cm}^{-1}$  assigned to binding vibrations of secondary amine and aliphatic amines, respectively. Hence, FTIR analysis confirmed the occurrence of capping protein around the AgNPs synthesised from culture supernatant of *P. stutzeri*. The presence of protein cap in nanoparticles help in stabilisation and binding to cell surface receptor results in increased binding and uptake of drug or genetic material on human cells [43, 44].

### 3.6 Antibacterial Assay of AgNPs against MDR *E. coli* and *K. pneumoniae*

Antibacterial assay of biosynthesised AgNPs was studied against MDR pathogenic strains (clinical isolates obtained from AIIMS, New Delhi) of *E. coli* and *K. pneumoniae* using disc diffusion method and zone of inhibition was depicted in Fig. 7. The results revealed that AgNPs were most effective against MDR *E. coli*. It was  $19\text{ mm}$  at  $2\text{ }\mu\text{g}/\text{disc}$ . For MDR *K. pneumoniae*, however, AgNPs showed a mild growth inhibitory effect of  $17\text{ mm}$  even at  $2\text{ }\mu\text{g}/\text{disc}$  when compared with MDR *E. coli* strain (Table 2). MDR *E. coli* and *K. pneumoniae* did not show zone of inhibition against ampicillin used as control (Fig. 7a).

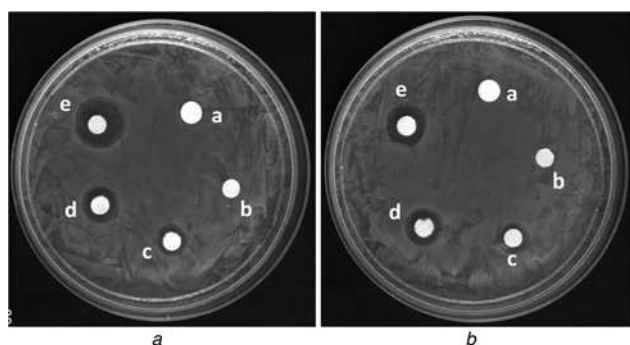


Fig. 7 Zone of inhibition of AgNPs at (c)  $0.5\text{ }\mu\text{g}$ , (d)  $1\text{ }\mu\text{g}$  and (e)  $2\text{ }\mu\text{g}$  (e) against (A) *E. coli* and (B) *K. pneumoniae*. (a) Ampicillin was used as positive control for MDR *E. coli* and *K. pneumoniae*. (b) Water was used as negative control

Furthermore, antibacterial activity of AgNPs in suspension was evaluated by counting CFUs of *E. coli* and *K. pneumoniae* after 1 h exposure to AgNPs ( $0.5\text{--}2\text{ }\mu\text{g}/\text{ml}$ ). Fig. 8 shows that increasing concentration of AgNPs significantly augments the kill percentage against *E. coli* and *K. pneumoniae*. Ag<sup>+</sup> from AgNPs can anchor to the negatively charged bacterial cell wall lead to perforation and results in cell lysis. Moreover, role of free radicals produced by AgNPs in contact with bacteria was also reported as demonstrated by electron spin resonance spectroscopy [10].

### 3.7 In vitro cytotoxicity of AgNPs

To explore the cytotoxicity of AgNPs, the MTT assay was employed measuring the mitochondrial activity of HeLa cells. Cells were exposed to increasing concentrations of AgNPs ( $0.5\text{--}2\text{ }\mu\text{g}/\text{ml}$ ) for 24 h. The MTT results of AgNPs did not show

Table 2 Zone of inhibition of AgNPs produced by the *Pseudomonas* sp. against MDR *E. coli* and *K. pneumoniae*

Sl. No	MDR bacterial strains	Zone of inhibition, mm $\pm$ scanning electron microscopy (SEM)		
		$0.5\text{ }\mu\text{g}/\text{disc}$	$1\text{ }\mu\text{g}/\text{ml}$	$2\text{ }\mu\text{g}/\text{disc}$
1.	<i>E. coli</i>	$11 \pm 0.4$	$16 \pm 0.8$	$19 \pm 1.1$
2.	<i>K. pneumoniae</i>	$7 \pm 0.5$	$11 \pm 0.6$	$17 \pm 0.9$

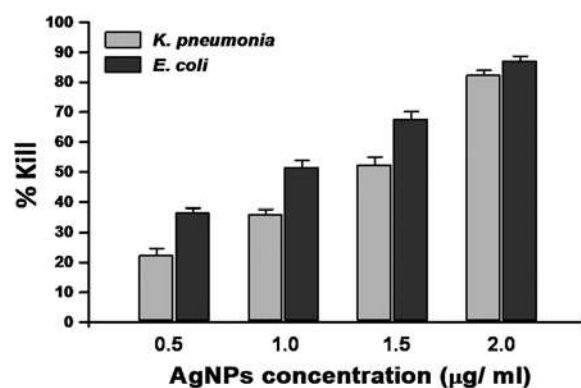
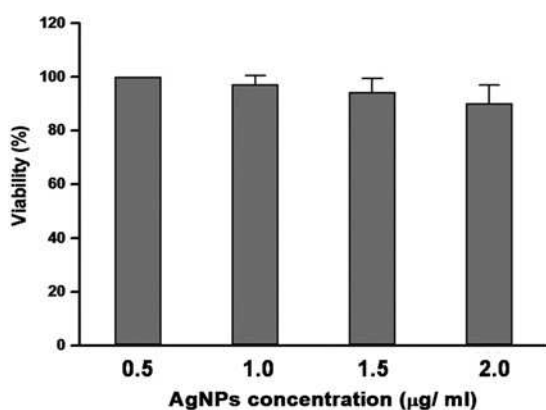


Fig. 8 Kill percentages of AgNPs



**Fig. 9** Concentration-dependent cytotoxicity of AgNPs

**Table 3** Hemolytic activity of AgNPs on RBCs

Sl. No	Treatment	Percentage hemolysis $\pm$ SEM
1.	control RBCs	2.3 $\pm$ 0.03
2.	RBC+1% triton $\times$ 100	100 $\pm$ 0
3.	0.5 $\mu$ g/ml	3.58 $\pm$ 0.1
4.	1 $\mu$ g/ml	4.7 $\pm$ 0.08
5.	2 $\mu$ g/ml	8.2 $\pm$ 0.11

concentration-dependent effects on the mitochondrial activity of HeLa cells (Fig. 9), indicating that the cells were unaffected by AgNPs synthesised using *P. stutzeri* at any of the employed concentrations. The selective toxicity of AgNPs toward bacterial cells as compared with HeLa cells can be explained in terms of the presence of transporter systems that may be responsible for differential binding and uptake of protein-capped AgNPs conferring cytotoxicity [47].

### 3.8 In vitro hemolytic of AgNPs

Hemolytic activity of AgNPs was performed to evaluate the biocompatibility of the AgNPs on normal cells, especially RBCs. The hemolytic assay was performed using different concentrations of AgNPs (0.5–2  $\mu$ g/ml) on RBCs. The absorbance values of RBCs in PBS and in 1% triton X-100 was used along with the values of RBCs treated with AgNPs on the formula. It was found that AgNPs exhibit little hemolytic activity against RBCs at 2  $\mu$ g/ml concentrations (Table 3). Our data are in correlation with the previous study done by Balaji *et al.* who reported that AgNPs (125  $\mu$ g/ml) synthesised using aqueous extract of *Rosa damascena* petals did not show hemolytic activity against RBCs [48].

## 4 Conclusion

This is the first report on biological synthesis of AgNPs using *P. stutzeri*, isolated from textile soil. The optimisation of reaction parameters resulted in monodispersed spherical AgNPs of size 8–10 nm. TEM was used to study the morphology, shape and size of biosynthesised nanoparticles. FTIR revealed the presence of protein cap on biosynthesised AgNPs. AgNPs exhibited excellent antimicrobial property on MDR *E. coli* and *K. pneumoniae*. This eco-friendly and cost-effective extracellular biosynthesis of naturally protein-capped AgNPs with potent antimicrobial activities from *P. stutzeri* has the potential to be utilised on a large scale for medical application.

## 5 Acknowledgment

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