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ORIGINAL RESEARCH

Hydrothermal assisted biosynthesis of silver nanoparticles from *Streptomyces* sp. GUT 21 (KU500633) and its therapeutic antimicrobial activity

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Abstract In the present investigation we report unique biological green synthesis of nanoparticles (AgNPs) by secondary metabolites of *Streptomyces* supernatant extract acting as reducing agents in hydrothermal process. Various divergent techniques like sonication, microven, heating and hydrothermal (autoclaving) techniques were employed to produce silver nanoparticles through microbe-mediated assistance. The *Streptomyces* sp. GUT 21 was isolated from the field soil sampled neighbouring the campus of Gulbarga University. Morphological and biochemical characterization of the strain was performed and its taxonomical genus identification was determined by 16s rDNA technique. The formation of nanoparticles was first monitored by measuring the surface plasmon resonance (SPR) band at 410 nm through UV–Visible absorption spectroscopy. FTIR analysis revealed that many efficient clusters of functional biomolecules are playing significant role in capping and synthesis process during hydrothermal method. The crystalline structure of the AgNPs and the presence of elemental silver nanoparticles were confirmed by powder X-ray diffraction (PXRD) and scanning electron microscopy. Our results indicated that, nanoparticles are spherical in shape with an average of 23–48 nm in size. The biosynthesized AgNPs exhibited significant antibacterial activity against *Escherichia coli* (MTCC 9537), *Klebsiella pneumoniae* (MTCC 109), *Pseudomonas aeruginosa* (MTCC1688) and *Staphylococcus aureus* (MTCC 96). This biotechnological development of synthesis of nanoparticles can further be exploited as "new-generation of antimicrobials" against multi-drug resistant bacteria (MDR) for various medical diagnostic applications.

Keywords *Streptomyces* · Autoclaving · Secondary metabolites · Silver nanoparticles (AgNPs) · Bactericidal activity

Abbreviations

SPR	Surface plasma resonance
FTIR	Fourier transform infrared spectroscopy
PXRD	Powder X-ray diffraction
SEM	Scanning electron microscopy
MDR	Multi drug resistant

Background

In recent times, nanotechnology and these miniature nano-sized particles are gaining significant prominence in biomedical and scientific arena. Previous studies have been reported on the use of diverse microorganisms in the biological production of nanoparticles with a significant potential for development [1, 2]. Several methods (chemical, physical or biological) have been established for the synthesis of these nanomaterials. Microbial synthesis of nanoparticles is nowadays prevailing high due to their eco-friendly, clean, reliable and rapid way of formation. Exploitation of biological assets such as microorganisms and their secreted by-products utilization has been suggested as possible environmental alternatives over these chemical and physical methods [3]. These biosynthesised

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nanoparticles are progressively been employed in wide range of applications in science, technology and medicine because of their distinctive optical and antimicrobial properties.

Soil is a natural habitat for Actinomycetes family which are typically branded due to their "earthy smell" nature. Since several decades, actinomycetes continue to serve as an important source of various kinds of antimicrobial metabolites. It has been estimated that 75 % of naturally occurring antibiotics have been produced by the members of genus *Streptomyces* and predominance of this genus in soil are tolerant to metal accumulation and acidic conditions [4]. The genus is also well known for its unique potential ability in production of wide variety of secondary metabolites, such as antibiotics, immunosuppressors and many other biologically active compounds used in various pharmaceuticals and commercial industries [5]. Exploitation of this *Streptomyces* genus in nanotechnology has recently received considerable attention [6]. It has been previously reported that amongst various other actinomycetes genera, *Thermomonospora* and *Rhodococcus* spp. [7, 8] also have the potency to bio-fabricate the metal ions especially gold and silver into several well-defined shape and sizes. In the present study, biosynthesis of silver nanoparticles were achieved using cell free supernatant extract (containing secondary metabolites) from a newly isolated bacterial *Streptomyces* sp. GUT 21 strain by different methodologies. The secondary metabolites of the *Streptomyces* sp. GUT 21 reacted with $AgNO_3$ present in the reaction solution, under the influence of high temperature and pressure (15 lbs) becomes more accessible for the silver ions to interact with all the available functional groups during autoclaving. Further, morphological characterizations of the synthesized nanoparticles like peak spectra, functional groups, shape and size of the nanoparticles were characterized by UV–Visible spectroscopy, infrared spectroscopy, X-ray diffraction, SEM and atomic force microscopy (AFM) analysis. Besides this, significant antimicrobial activity of silver nanoparticles was evaluated against multi-drug resistant (MDR) organisms like *Escherichia coli* (MTCC 9537), *Klebsiella pneumoniae* (MTCC 109), *Pseudomonas aeruginosa* (MTCC1688) and *Staphylococcus aureus* (MTCC 96) with determination of MIC values measured in liquid cultures.

Results and discussion

Isolation and taxonomical identification of *Streptomyces* sp. GUT 21

Isolation and characterization of *Streptomyces* sp. GUT 21 strain was achieved on SCA medium. Morphological resembling of aerial and branched mycelia (Fig. 1) of the isolate was examined under bright field microscopy. Biochemical characterization and carbon source utilization tests were as summarized in (Table 1). SEM images examined of this strain indicated that the isolate possessed substrate mycelia and extensively branched aerial hyphae that further differentiated into smooth surfaced spores

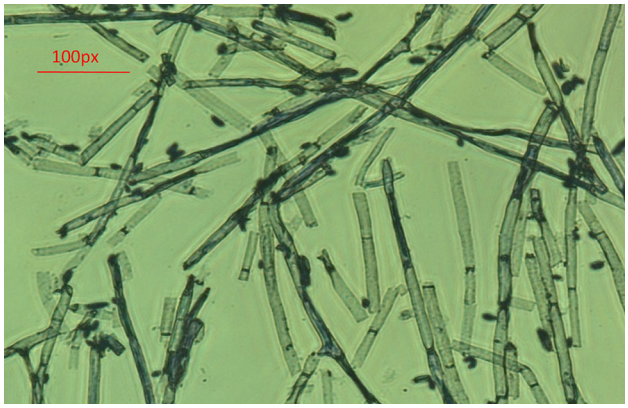

Fig. 1 Bright field microscopy of Aerial mycelia and spore of *Streptomyces* sp. GUT 21 strain observed under ×100 magnification, NIKON SERIES E110448. *Scale* 100px

Table 1 Biochemical characteristics and carbon source utilization features of the isolated strain *Streptomyces* sp. GUT 21 from Gulbarga, Karnataka region

Characteristic features	*Streptomyces* sp. GUT 21
Biochemical	
Gram staining	Positive
Starch hydrolysis	Positive
Casein hydrolysis	Positive
Gelatin hydrolysis	Negative
Indole production	Negative
Methyl-red test	Negative
Voges-proskaur test	Negative
Catalase test	Positive
Oxidase test	Positive
Urea hydrolysis	Positive
Nitrate reduction test	Positive
H_2S production	Negative
Citrate utilization test	Negative
Carbon source utilization	
Glucose	+++
Mannitol	−
Raffinose	−
Sucrose	+
Galactose	++

+++ Strong positive, utilized; ++ positive, utilized; + weakly positive, utilized; − negative, not utilized

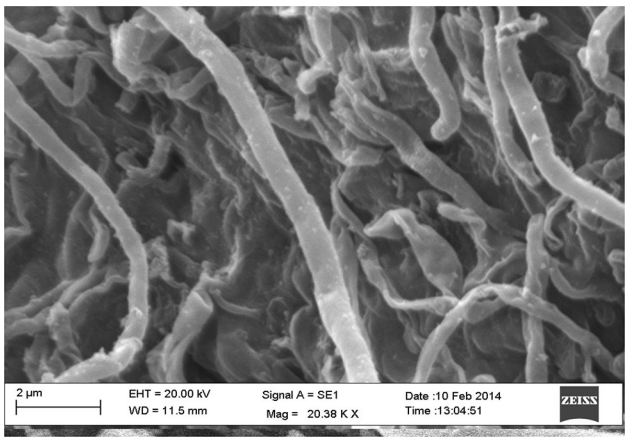

Fig. 2 Scanning electron micrograph of mycelia of *Streptomyces* sp. GUT 21 strain grown on ISP2 medium for 5 days at 28 °C. *Bar* 2 µm

(Fig. 2). The 16S rRNA gene is the most commonly used marker for inferring the phylogenetic relationship among bacterial species due to its highly conserved nature [9]. Thus, in the present study, the acquired 1377 bp DNA sequence of the isolate coding 16S rRNA gene was deposited at GenBank database (NCBI) under the Accession No KU500633. Consequently, based on 16S rDNA sequence analysis, the strain exhibited 98 % homology with that of available *Streptomyces* sequences (Fig. 3).

In this study, we have reported the potentiality of *Streptomyces* sp. for the biosynthesis of silver nanoparticles with extracellular method by means of screening different approaches (Fig. 4). The development of brownish yellow colour was only observed in hydrothermal (autoclaving) method. However, in microwave and heating methods, colour change was observed (might be due to irradiation of precursor silver nitrate chemical) but no detection of SPR band was observed. The formation of silver nanoparticles can be primarily distinguished through physical visible observation in the colour change of solution during the reaction from colourless to pale yellow [10]. This is the first time exciting report that microbe-mediated synthesis of nanoparticles was carried out from *Streptomyces* sp. by hydrothermal autoclaving process in microbial system as compared with the previous report synthesized from plant gum ghatti *Anogeissus latifolia* [11]. In UV–Visible spectra a strong peak of SPR band with a maximum at 410 nm (Fig. 5) was observed in autoclaving method which corresponds to typical plasmon resonance of oscillating and conducting electrons on the surface of silver nanoparticles. This band was a sign for formation of spherical nanoparticles in a reaction solution. Also, Fig. 6 evidently depicts the size distribution of synthesized AgNPs and AgNPs were distributed in the range of 20–224 nm with the average particle diameter of 44 nm. The polydispersity index (PdI) was found to be 0.426. The DLS results confirmed the broad distribution of AgNPs, which was previously supported by UV–visible spectra and further confirmed by atomic force microscopy.

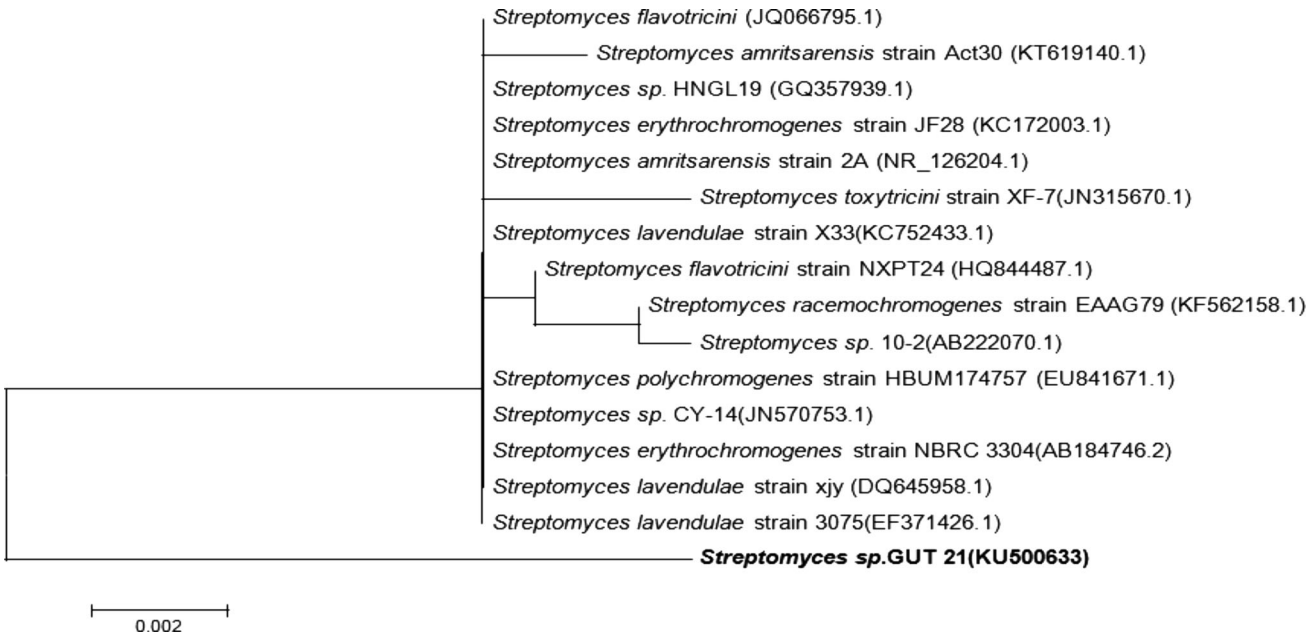

Fig. 3 Phylogenetic analysis of *Streptomyces* sp. GUT 21 (KU500633) strain constructed using the neighbour-joining method with the assist of MEGA 6.0 version software. *Bar* 0.002 represents the substitutions per nucleotide position indicating the evolutionary genetic distance. The 16S rDNA sequence obtained was mapped by BLAST hit similarity at the NCBI database showing 98 % homology with that of available *Streptomyces* sequences

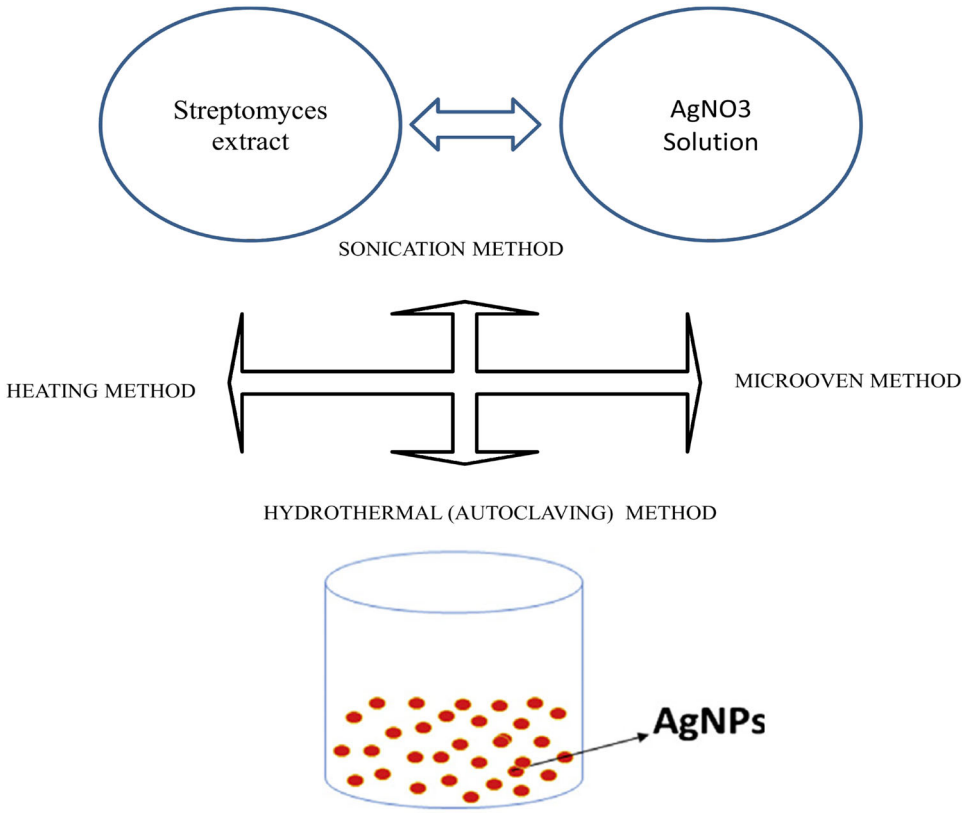

Fig. 4 Schematic graphical representation of biosynthesis of nanoparticles by different methodologies. The cell free supernatant extract containing secondary of *Streptomyces* sp. GUT 21 used for various divergent techniques like sonication, microven, heating and hydrothermal (autoclaving) techniques to produce silver nanoparticles

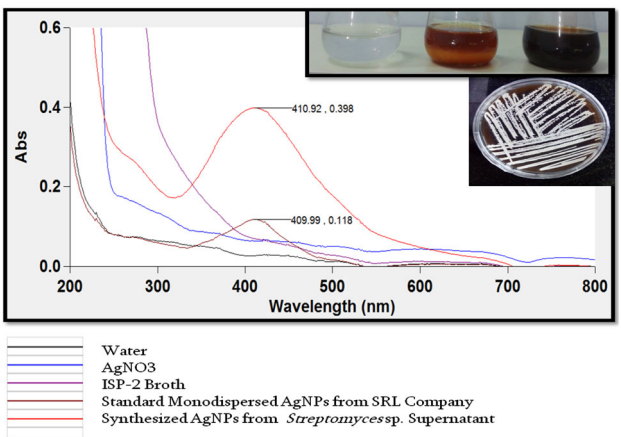

Fig. 5 UV–Vis absorption peak of AgNPs synthesized by using cell free supernatant extract of *Streptomyces* sp. GUT 21. Development of brownish yellow colour and bioreduction of silver ions Ag^{+} to Ag^{0} of AgNPs by physical change to *dark brown colour* detected in hydrothermal autoclaving method. *Inset* picture of *Streptomyces* sp. GUT 21 sub-cultured on SCA medium

FT-IR investigations of silver nanoparticles

FTIR molecular fingerprint spectrum of the freeze-dried samples were obtained in the wavelength range from 500 to 4000 cm^{-1} to identify the possible functional groups involved in the bioreduction and capping of silver nanoparticles. The spectra were recorded for the *Streptomyces* sp. GUT 21 supernatant extract before (Fig. 7a) and after reacting with silver nitrate (Fig. 7b) in hydrothermal autoclaving process. Generally, peaks at 3435.9 and 3434.2 cm^{-1} are corresponding to O–H stretching vibrations of alcohols and phenolic group of compounds. The peaks at 2920.8, 2850.7 cm^{-1} and the peaks at 2924.8, 2851.50 cm^{-1} in case of AgNPs were attributed to the stretching and bending vibration of C–H in alkanes. A single peak observed at 1600.43 cm^{-1} corresponds to amide linkage and bending vibration of N–H groups of proteins, respectively where as in case of AgNPs spectra, the peaks observed at 1750, and 1631 cm^{-1}, represents the C=O stretch, –C=C stretch of esters and amide group respectively, which corresponds to heterocyclic compounds like proteins. This serves as support for proteins present in the supernatant extract as capping agents for the biosynthesized AgNPs [12, 13]. It has been reported that proteins may bind to the nanoparticles either with the free amine groups or cysteine residues and cap the nanoparticles [8]. Also, the genus *Streptomyces* are well-known to produce an array of bioactive compounds with diverse biological properties [14]. The FTIR spectra of *Streptomyces* sp. GUT 21 supernatant extract after the reaction with silver nitrate assisted by hydrothermal process is shown in Fig. 7b. The comparison between both the spectra

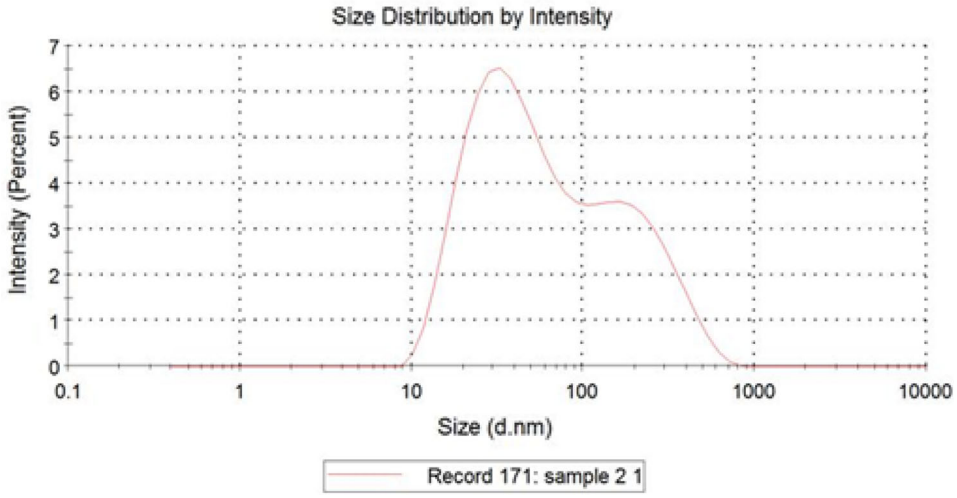

Fig. 6 DLS particle size distribution image of silver nanoparticles. *X*-axis and *y*-axis represents size (d nm) and intensity (%), respectively

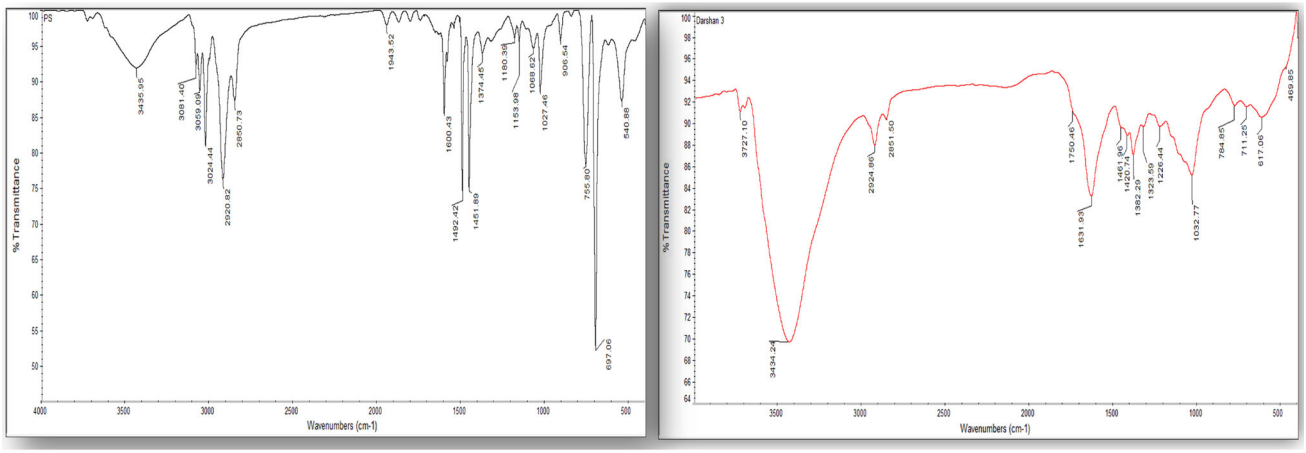

Fig. 7 FT-IR spectrum of **a** *Streptomyces* sp. GUT 21 supernatant extract containing secondary metabolites, **b** Hydrothermally biosynthesized AgNPs spectra, performed in the diffuse reflectance mode blended in KBr pellets. The *graph* depicts spectra (cm^{-1}) on *x*-axis and transmittance (%) on *y*-axis

illustrates the broad peak shift and decrease in the intensity in Fig. 7b, which can be attributed to the role of bio molecules like esters, proteins and amides in the biosynthesis of AgNPs. The appearance of new peaks at 1420–1226 cm^{-1} represent the C–H stretching vibrations, N–H bending, $–CH_3$ wagging and C–OH stretching vibrations whereas the sharp peaks appeared at 1032 and 711 cm^{-1} represent the C–O stretching and aromatic –CH deformation respectively. Based on both the spectrum fingerprint, in the present study, it can be inferred that many functional groups are involved in the conversion of silver ions to silver nanoparticles. The disappearance of the band or intensity decrease, such as the band at 1461 and 469 cm^{-1} can also be attributed to the reduction of silver ions. The proteins present in the supernatant extract are believed to cap, stabilize and prevent the aggregation of AgNPs. In addition, other efficient cluster of bioactive molecules like powerful reducing aldehyde groups along with the existing amine groups reduce more and more of silver ions to elemental silver. Also, previously reported, elevated temperature accelerates the reduction process by aldehydes in case of green synthesised AgNPs using soluble starch as stabilising agent [15]. Further, these nanoparticles are probably capped and stabilized by the complex compounds which are secreted by *Streptomyces* genus during its life cycle development [16]. The reduction of colloidal silver ion by this supernatant extract was not observed at room temperature. Thus, an external source of energy was provided for biosynthesis of nanoparticles, to occur in the form of hydrothermal autoclaving process.

It was perceived that autoclaving this solution at 121 °C temperature and 15 lbs of pressure increased the synthesis

and stabilisation of nanoparticles. It is known that elevated temperature and pressure accelerate the synthesis of nanoparticles [17]. We found, that the possible mechanism could be, autoclaving which is a slow and a steady process wherein there is a gradual increase in temperature from 0 to 121 °C, this process steadily accelerate the synthesis of nanoparticles by jumping of free Ag^{+} ions from lower energy state (electrons) into higher energy state thereby binding and embedding with free secondary metabolites functional groups present in the reaction solution. In addition, autoclaving procedure completely reduces other microbial contamination perhaps attained during usage and transportation of nanoparticles synthesis. Subsequently, Chun et al. have also reported that NADH-dependent enzymes play a key role in these biosynthetic and bio-transformation reactions as an electron carrier [18]. Yet, the mechanism underlying the biosynthesis of AgNPs by microorganisms needs to be elucidated in detail.

Nanoparticle morphological and structural analysis

The average size and crystalline nature of biosynthesized AgNPs were obtained by Powder X-ray diffraction peaks (Fig. 8). The peaks obtained at 2θ angle = 38.20°, 44.40°, 64.60° and 77.60° corresponding lattice plane values were indexed as (1 1 1), (2 0 0), (2 2 0) and (3 1 1) reflections planes of a faced centre cubic (fcc) synthesized silver nanocrystal, respectively (Ag XRD Ref. No. 00-004-0783) [19]. The average crystallite size (D) was estimated from the line broadening diffractogram in PXRD using Debye Scherer's formula $D = \frac{K\lambda}{\beta\cos\theta}$ and was found in the range of 15–20 nm. The SEM and AFM images further provided information regarding the morphology of the synthesized nanoparticles that confirmed the existence of very small and uniformly shaped spherical nano particles (Figs. 9, 10). These hydrothermally biosynthesized nanoparticles are spherical in shape and polydispersed. It was observed that larger particles of AgNPs are formed due to aggregation of nano particles which might be induced by the evaporation of solvent during sample preparation incase of SEM and the difficulty of precise cantilever tip attachment to the exact sample end during AFM set up. This could have added for the discrepancy and variation in particle size. Thus, it can be attributed that synthesized nanoparticles are in the range of 23–48 nm in size with some nanospheres in the regime of 15–20 nm.

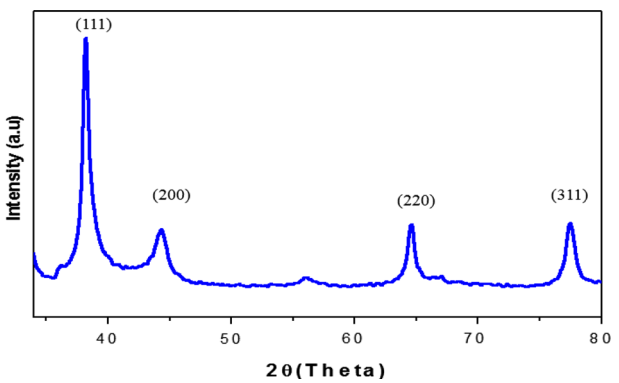

Fig. 8 X-ray diffraction patterns of Ag-NPs biosynthesized using cell free supernatant extract containing secondary metabolites of *Streptomyces* sp. GUT 21

Antimicrobial activity of AgNPs

A significant antimicrobial activity of silver nanoparticles was achieved against both gram classes of multi-drug resistant (MDR) organisms (Figs. 11, 12). In case of agar well diffusion assay, the zone of inhibition was expressed as the average mean ± SD by taking three replicates. The zone of inhibition was measured for gram positive pathogens like *S. aureus* (MTCC 96) and gram negative organisms like *E. coli* (MTCC 9537), *K. pneumoniae* (MTCC 109) and *P. aeruginosa* (MTCC1688) respectively depicted in Table 2. After 24 h of incubation, in case of gram positive bacteria *S. aureus,* it can be seen that the zone of inhibition (ZOI) was increased with an increase in AgNPs concentration. The maximum inhibition of 28 mm was observed with a dosage of 45 µg/mL of AgNPs against *K. pneumonia* whereas, the inhibition against *E. coli* showed 26 mm with 30 µg/mL of AgNPs concentration. Discretely, in case, of *P. aeruginosa* less inhibition was observed with a sensitization reaction around the AgNPs loaded wells. The ZOI acquired around positive control (Nalidixic acid) was less in diameter compared to AgNPs. This proved the significant antibacterial efficacy of synthesized AgNPs. Previously, Muthukumar et al. have also reported the similar results in case of ultrasound assisted green synthesis of silver nanoparticles using weed plant [20]. Similarly, different concentrations of *Streptomyces* sp. GUT 21 extract and control residual silver ion were also tested against all the pathogens, which showed very less zone of inhibition around the wells (data not shown). The exact mechanism involved in which silver nanoparticles exhibit to show antimicrobial effect is not clearly understood and is unclear. However, there are conversely various assumptions that silver ions have capacity to inhibit the bacterial replication, by binding and denaturing bacterial DNA [21, 22]. Formerly, a group of researchers have also reported the bactericidal potential of silver ions against *S. aureus* and *E. coli* which have been known to bind with negatively charged bacterial cell wall resulting in rupture and consequent denaturation of proteins which leads to cell death [23]. Antimicrobial activity of AgNPs may also be attributed to its special characteristics because of small size

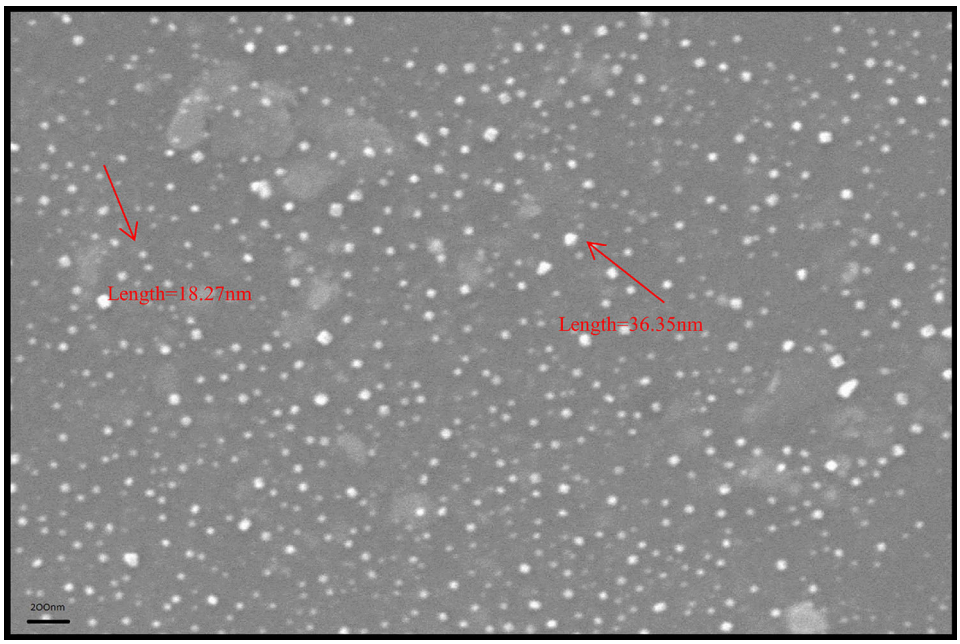

Fig. 9 SEM image of biosynthesised AgNPs synthesised using *Streptomyces* sp. GUT 21 at a *scale bar* of 200 nm. Dry powdered AgNPs sample of nanoparticles solution was homogenised with distilled water. A small drop of this sample was coated on a thin layer of platinum grid and allowed to dry. Morphological image dimensions of AgNPs were captured on a JEOL JSM-7600F (USA) instrument accomplished at an accelerating voltage of 15 kV

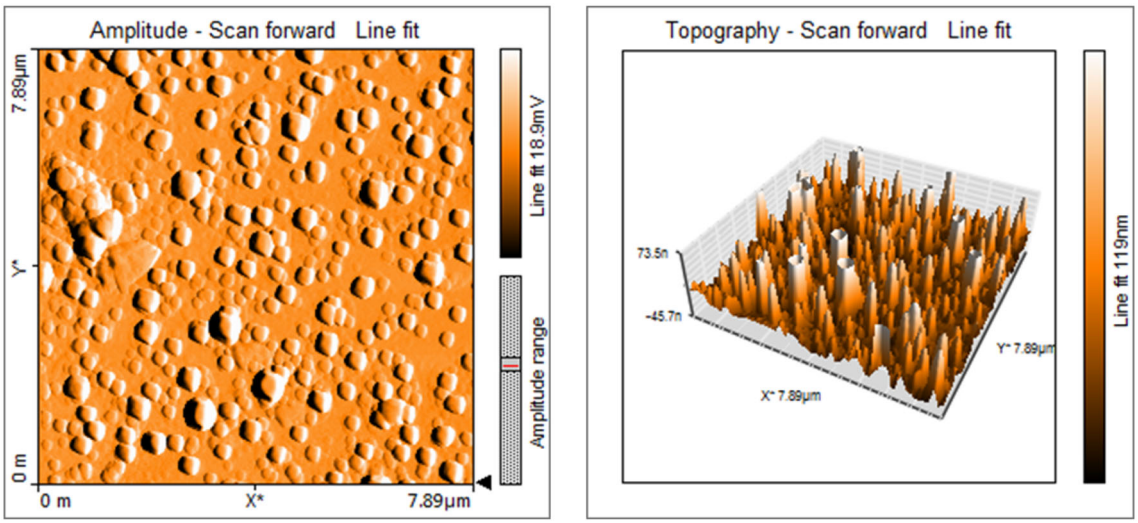

Fig. 10 AFM images of biosynthesised AgNPs. Left prospective image shows evenly spread spherical nanoparticles along with the *top* view three dimensional symmetrical images of AgNPs. AFM morphological analysis were executed using an AFM Explorer microscope (Thermo Microscopes, USA) in room temperature, with a non-contact mode through Si cantilevers of a 1650-00 type (Thermo Microscopes) with a nominal tilt radius of 10 nm and reverberating frequencies of about 220 kHz. The image measurements were calculated by using the SPM Lab software

and high surface area to volume ratio [24]. Nevertheless, due to emergence of multi drug resistance (MDR) developed by various organisms, serious challenges have been mounted on researchers to pave a way to identify novel antimicrobial agents. Therefore, to overcome this drug resistance of microorganism, it is essential to discover different approaches of biosynthesising nanomaterials which may act as surface coating agents in medical diagnostic applications to hinder the microbial growth.

Determination of minimal inhibitory concentration (MIC) of AgNPs

Minimal inhibitory concentration (MIC) of biosynthesised AgNPs (Table 3) was assessed against both classes of organisms. The silver nanoparticles exhibited lowest minimum inhibitory concentration (MIC) against *E. coli* (MTCC 9537) at 14 µg/mL, *K. pneumoniae* (MTCC 109) at 12 µg/mL and *P. aeruginosa* (MTCC1688) at 10 µg/mL.

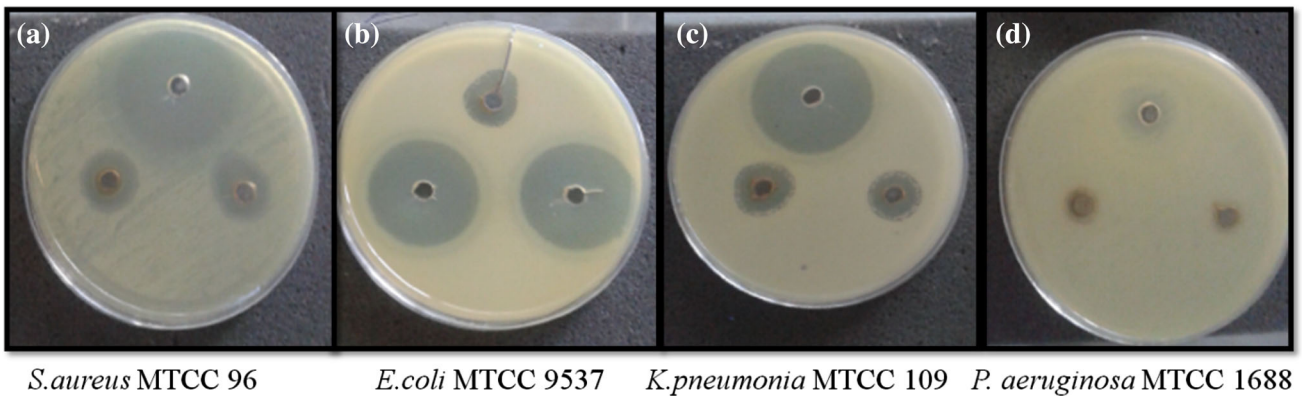

Fig. 11 Antimicrobial activity of biosynthesized AgNPs by *Streptomyces* sp. GUT 21 against pathogenic organisms'. **a** *Staphylococcus aureus* (MTCC 96), **b** *E. coli* (MTCC 9537), **c** *K. pneumoniae* (MTCC 109), **d** *P. aeruginosa* (MTCC1688)

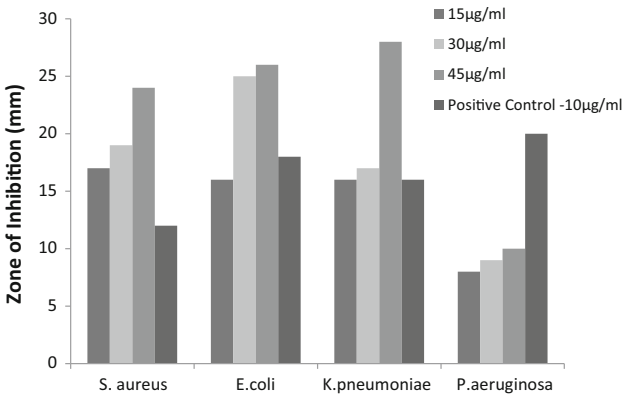

Fig. 12 Antibacterial activities of various concentrations of silver nanoparticles (15–45 µg/mL). The results are expressed as the mean ± SD of triplicates. Nalidixic acid (10 µg/mL) was used as a control

In case of gram positive organism like *S. aureus* (MTCC 96), the MIC value was recorded as 15 µg/mL. Previously, in batch studies with *E. coli* and colloidal silver nanoparticles (size range 2–25 nm) MIC was reported to be in the range of 3–25 µg/mL for initial bacterial concentration 10^5–10^8 CFU ml^{-1} [25, 26]. We have found similar results in case of *E. coli* culture indicating bactericidal efficiency of biogenic nanoparticles. Compared to copper nanoparticles, silver nanoparticles depict higher sensitivity to both *E. coli* and *S. aureus;* however the difference is less for *S. aureus* compared to *E. coli* [27] also depicting similar values in this study (Table 2). However, Kim et al. [28] have reported that gram positive *S. aureus* is more resistant to silver nanoparticles compared to gram negative *E. coli* based on the studies with single strains of each culture. Moreover, in our study, we demonstrated that in case of *Klebsiella* and *Pseudomonas*, the MIC values were slight discrete as 12 and 10 µg/mL compared to previously published data of Pourmand et al. in their tested pathogenic strains [29]. This might be due to variation in the size of particles, biosynthesis process and initial bacterial concentration, direct comparison between the broth dilution assay and well diffusion studies was not feasible. Conversely, there are reports in the literature which show that electrostatic attraction between negatively charged bacterial cells and positively charged nanoparticles is crucial for the activity of nanoparticles as antimicrobial agents [30]. Hence, the antibacterial activity of AgNPs is not merely dependent on the structure of bacterial membrane although we found similar correlation between the inhibition zones observed in agar well diffusion test and MIC values determined in liquid cultures.

Conclusion

The present investigation has revealed the biosynthesis of AgNPs utilizing secondary metabolites of *Streptomyces* sp. GUT 21 through a single step hydrothermal process with

Table 2 Mean diameter zone of inhibition (mm) of biosynthesised AgNPs against *E. coli* (MTCC 9537), *K. pneumoniae* (MTCC 109), *S. aureus* (MTCC 96), and *P. aeruginosa* (MTCC1688) by taking three replicates

Microorganisms	Mean zone of inhibition (mm)			Positive control Nalidixic acid 10 µg/mL
	15 µg/mL	30 µg/mL	45 µg/mL	
Escherichia coli (MTCC 9537)	16.25 ± 2.09	26.75 ± 1.50	27.05 ± 3.20	18.10 ± 1.50
Klebsiella pneumoniae (MTCC 109)	16.25 ± 2.60	17.20 ± 2.09	28.50 ± 2.60	16.50 ± 3.60
Staphylococcus aureus (MTCC 96)	17.50 ± 1.50	19.25 ± 1.50	24.25 ± 2.09	12.25 ± 3.20
Pseudomonas aeruginosa (MTCC1688)	8.05 ± 3.60	9.05 ± 3.20	10.05 ± 3.60	20.05 ± 2.60

Table 3 Minimum inhibitory concentration (MIC) of the AgNPs against various bacterial pathogens

Microorganisms	Minimum inhibitory concentration	
	AgNPs (μg/mL)	Antibiotic (μg/mL) Nalidixic acid
Escherichia coli (MTCC 9537)	14	11
Klebsiella pneumoniae (MTCC 109)	12	9
Staphylococcus aureus (MTCC 96)	15	13
Pseudomonas aeruginosa (MTCC1688)	10	8

The data is presented as the mean ± value standard deviation of three replicates

promising fast kinetics and no involvement of hazardous substances. In addition, this could be a new direction to bio fabricate nanoparticles with better controlling of size from other Actinomycetes genera and can be an alternative route over the physical and chemical methods. The biosynthesized nanoparticles were characterized by UV–Visible, infrared spectroscopy, X-ray diffraction, SEM and atomic force microscopy. FTIR fingerprint confirmed the utility of *Streptomyces* extract acting as capping and stabilizing agents during autoclaving process to prevent further aggregation and agglomeration of AgNPs. The synthesised AgNPs exhibited tremendous antimicrobial activity against the tested bacterial pathogens. This biotechnological advancement of synthesis of nanoparticles can articulate a new way in bio prospecting which can enhance the biological, medical and disease diagnostic applications. However, it would be most essential to recognize their potential reciprocal action and optimized dosage formulation of biosynthesized nanoparticles before their use in nano-technological applications.

Methods

The soil samples were collected from the nearby fields on the campus of Gulbarga University; Gulbarga (latitude of 17.3130°N-longitude of 76.8743°E) Karnataka Province, India. The samples were collected in aseptic polystyrene bags and isolation of actinomycetes was done by serial dilution and spread plate technique [31].The actinomycete strain GUT 21 was isolated in SCA (Soluble starch, 10 g/L, casein 0.3 g/L, KNO_3 2 g/L, NaCl 2 g/L, K_2HPO_4 2 g/L, $MgSO_4{\cdot}7H_20$ 0.05 g/L, $CaCO_3$ 0.02 g/L, $FeSO_4{\cdot}7H_2O$ 0.01 g/L, Agar 18 g/L, pH 7.2) medium and incubated at 28 °C for 7 days.Morphological and biochemical characterization of this strain was performed by following the method of Shirling and Gottlieb [32]. Morphology was studied in both bright field (NIKON SERIES E110448) and scanning electron microscopy (SEM model, ZEISS 505, USA). Utilization of various carbon sources was assessed on a minimal media containing M9 salts.

In order to determine its taxonomical identity of this isolate, molecular characterization was performed by using universal 16S rRNA primer pair of 27F and 1492R [33]. The PCR amplicon products were analysed in 1 % agarose gel by electrophoresis and purified by using PCR purification kit (QIAGEN). The 16S rDNA gene sequencing was outsourced at a sequencing facility available at XCELERIS Labs (Ahmadabad, India).The DNA sequence obtained was then mapped by BLAST hit similarity at the NCBI database and the sequence was deposited in Gene Bank with an Accession number KU500633. The 16S rRNA gene sequence of *Streptomyces* sp. GUT 21 strain was aligned with reference sequences retrieved from GenBank and the Phylogenetic tree was constructed using the neighbour joining method with MEGA 6 software [34].

Culture conditions and bio-synthesis of AgNPs by different methodologies

To acquire cell free supernatant extract, a loop full culture of *Streptomyces* sp. GUT 21 was inoculated in soybean meal medium for production of secondary metabolites [35]. The culture flask was incubated at 28 °C for 10 days at 200 rpm under shaking conditions. Further, this culture broth was centrifuged at (13,000×*g*, 10 min) at 4 °C to ensure complete removal of mycelia from bacterial biomass. The cell free supernatant extract containing secondary metabolites having pH 6.36 was collected carefully and used for facile biosynthesis of nanoparticles by different methodologies. A negative control reaction mixture was maintained of only precursor $AgNO_3$ and lone secondary metabolite supernatant solution was also taken in all different methodologies biosynthesis procedure.

Initially, four different methodologies like sonication, micro oven, heating and hydrothermal (autoclaving) were employed for biosynthesis of nanoparticles from *Streptomyces* sp. using above cell free supernatant extract (CFSE). In sonication process, powerful ultrasound radiations up to 30 kHz were applied to prepared solution to enhance the reaction with sonicator (Hielsher Company, UP100H) over a period of time like 10, 20 min up to 1 h until a physical

colour change visualisation. To monitor the silver nanoparticle synthesis, UV–Visible spectra was recorded at different time intervals. However, no colour change was observed and concluded that no nanoparticle synthesis occurred. In microwave method of synthesis, microwave radiations were introduced into the different reaction solutions with different time gap intervals. Microwaves with frequencies in the range of 300–300 GHz were used at different power rate of 300, 450, 600 and 800 W for 30 and 60 s for irradiation of nanoparticles to observe physical colour change and spectra was recorded for confirmation of nanosynthesis. Here, colour change was observed but no detection of SPR band was observed. Additionally, for synthesis of nanoparticles, two flasks containing 100 mL of prepared solution were also heated at 65 and 85 °C in a water bath to visualize for the colour change and feasible synthesis of AgNPs, for a period of 1 h and extended up to 2 h respectively. Nevertheless, in this process also no detection of peak occurred in UV–Visible spectral analysis. Lastly, in hydrothermal autoclaving method, 10 mL of the cell free supernatant extract (CFSE) extract was added to 500 mL of Erlenmeyer flask containing 1 mL of 100 mM $AgNO_3$ with final volume made up to 100 mL using distilled water and kept for autoclaving at 121 °C at 15 lbs pressure for 15 min. Additionally, this solution was also allowed to cool at room temperature under dark conditions. Further, colour change was monitored and a UV–Visible spectrum was recorded by scanning the wavelength range from 200 to 800 nm in spectrophotometer (Varian Cary 50 Bio UV–VIS Spectrophotometer).

Characterization of AgNPs

UV–Vis spectroscopy analysis

Biosynthesis of the nanoparticles (bioreduction of silver ions Ag^+ to Ag^0) AgNPs was virtually detected by physical change to dark brown colour in hydrothermal autoclaving method screened across various methods. Subsequently, for confirmation of nanoparticle synthesis, commercially available monodispersed silver nanoparticles (AG20) from SRL Company (catalogue no. 58322) were also used as a standard reference. UV–visible spectrometric measurements were performed on Varian Cary 50 Bio UV–VIS Spectrophotometer in the 200–800 nm range.

DLS analysis of silver nanoparticles

Dynamic light scattering of the biosynthesized AgNPs was done to calculate particle size and its distribution using Dynamic Laser Scattering with a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd.). This method was used to analyze the size of particles or molecules in Brownian motion suspended into solution. AgNPs samples were 1:10 diluted and were then examined for Z-average diameter (d nm), size distribution and polydispersity index (PdI).

FT-IR investigations of silver nanoparticles

FTIR chemical functional group analysis of *Streptomyces* sp. GUT 21 extract containing diverse secondary metabolites and hydrothermally synthesized AgNPs was performed on a NICOLET, USA (MODEL 6700) instrument in the diffuse reflectance mode at a resolution of 4 cm^{-1} blended in KBr pellets and the spectra were recorded in the wavelength interval of 4000 and 500 nm^{-1}.

PXRD measurements of silver nanoparticles

Powder X-Ray diffraction (PXRD) pattern was studied for the synthesized AgNPs using (Cu-Kα radiation source) using a 12 kW rotating Cu anode based Bruker (Smart Apex II) powder diffractometer (Model Small Apex-II) operating in Bragg–Brentano geometry. The scanning dimension (2Θ) value was selected from 20° to 80° angles. Finally, the average crystalline size (D) of the AgNPs was estimated from the line broadening in diffractogram using Debye Scherer's formula $D = \frac{K\lambda}{\beta \cos \theta}$.

Scanning electron microscopy examination of silver nanoparticles

Scanning electron microscopy was done to understand the morphological and elemental information of silver nanoparticles. Dry powdered AgNPs sample of biosynthesized silver nanoparticles solution was homogenised with distilled water. A small drop of this sample was coated on a thin layer of platinum grid and allowed to dry. Finally, image dimensions were captured on a JEOL JSM-7600F (USA) instrument accomplished at an accelerating voltage of 15 kV.

Atomic force microscopy of silver nanoparticles

Morphological characterizations of the biosynthesised AgNPs from above *Streptomyces* extract was also obtained by AFM technique. AFM analysis were executed using an AFM Explorer microscope (Thermo Microscopes, USA) in room temperature, with a non-contact mode through Si cantilevers of a 1650-00 type (Thermo Microscopes) with a nominal tilt radius of 10 nm and reverberating frequencies of about 220 kHz. Glass slides cleaned with sulphuric acid, hydrogen peroxide and deionized water were used as the substrate. For the sample preparation, thin films supplied with the instrument were kept for 24 h at room temperature

and then were submitted to a heat treatment at 80 °C for 2 h and then coated on glass slide. The image measurements were carried out using SPM Lab software which included levelling on the plane, background fine adjustments and intermittent stripe removing with fine cantilever adjustments.

Antimicrobial activity of AgNPs

Antimicrobial activity was investigated for hydrothermally biosynthesized AgNPs against bacterial pathogens consisting of gram-positive (*S. aureus* MTCC 96) and gram-negative (*E. coli* MTCC 9537, *K. pneumoniae* MTCC 109 and *P. aeruginosa* MTCC1688) origin adapting agar well diffusion method [36]. Test cultures of bacteria were grown in Nutrient broth at 37 °C in an incubator shaker at 160 rpm. These pathogenic test organisms were swabbed on Nutrient Agar (NA) plates. A sterilised cork borer with a diameter of 6 mm was impregnated into the agar medium. The 50 µL of (15, 30 and 45 µg/mL) the test AgNPs samples were loaded into each well and allowed to air dry completely. The effects of AgNPs were compared with the positive antibiotic (Nalidixic acid 10 µg/mL) and negative (supernatant extract) controls. The growth inhibition of bacterial pathogens was assessed by the corresponding zone of inhibition (ZoI) [37] after incubating the plates at 37 °C for 24 h. ZoI was measured by using a standard HiAntibiotic Zone scale^{TM} Himedia (Mumbai, India).

Determination of minimal inhibitory concentration (MIC) of AgNPs

The antimicrobial efficacy of silver nano particles (AgNPs) was examined using the standard broth dilution method (CLSI M07-A8). The MIC was determined in LB broth using Ag-NPs in concentrations ranging from 2, 4, 6, 8, 16 to 1024 µg/mL. Bacterial cells were grown in 10 mL LB broth by inoculating about 10^6 CFU/mL (O.D. 0.1 at 625 nm, 0.5 McFarland's standard) of respective cultures. The positive control was used as an antibiotic (Nalidixic acid 10 µg/mL) and negative control contained only inoculated broth. All test tubes were incubated at 37 °C for 24 h and measured the absorbance at 600 nm. The MIC is the lowest concentration value noted by the visual turbidity of the tubes both before and after incubation. In case of MIC assay, the bacterial inhibition was expressed as the average mean ± SD by taking a set of three replicates.

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