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RESEARCH ARTICLE

Extracellular fabrication of bio-nanostructures from *Ralstonia* sp. strain NS-7: Characterizations and their microbiological evaluation

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Abstract

Objectives: In this study, we took advantage of nanotechnology to systematically investigate the antimicrobial activity of silver nanoparticles (AgNPs) against pathogenic microorganisms. This study aimed to synthesize AgNPs from *Ralstonia* sp. strain NS-7 and further characterization of synthesized AgNPs. **Materials:** The molecular characterization of isolated strain *Ralstonia* sp. NS-7 was done by 16S rRNA gene sequencing and the characterizations of synthesized AgNPs was achieved by UV-Visible spectroscopy, AFM, FTIR, HR-TEM, SEM, EDS and XRD. Later on, the efficacy of previously synthesized AgNPs was assessed *in vitro* against pathogens, such as *Escherichia coli*, *Enterococcus faecalis*, *Streptococcus pneumoniae* and *Staphylococcus aureus*. **Finding:** The UV-visible spectrophotometric observation of synthesized AgNPs showed maximum absorbance at 420 nm, the AFM data revealed the polydispersity of spherical nanoparticles. Further, the FTIR analysis expressed a unique IR spectral band patterning and the HR-TEM and SEM analysis showed the size of bio-synthesized AgNPs in the range of 14.72 nm to 47.32 nm. The analysis of phylogenetic tree of the strain NS-7 revealed the most sequence similarity with *Ralstonia* sp. strain PGNP6. Finally, the AgNPs represented a broad-spectrum antimicrobial activity against gram-positive and gram-negative bacteria. **Application:** The biological method for the synthesis of AgNPs is eco-friendly, economical, green and non-toxic. Synthesized AgNPs from *Ralstonia* sp. strain NS-7 could be used as an alternative source of antimicrobial for the management of pathogenic and multi-drug resistant microorganisms.

Keywords: *Ralstonia* sp.; FTIR; HR-TEM; 16S rRNA gene sequencing; antimicrobial activity; AgNPs

1 Introduction

Nanotechnology is emerging as a highly developing branch with its application in various fields of science and technology for the purpose of synthesis of new materials

at nanoscale range [1–3]. Nanotechnology deals with the materials at nanometer (nm) scale; usually from 1 nm to 100 nm. There are many techniques, which have developed for synthesizing AgNPs. But each technique of synthesizing nanoparticles solely and primarily depends on any one of biological, chemical and physical methods [4]. The biological method of synthesis of nanoparticles has the greatest significance over the chemical and physical methods [5]. The biological nanoparticles can be produced from a large number of metals, such as silver, gold, platinum, zinc, copper, iron, titanium, aluminium, chromium, cobalt, nickel etc [6]. Despite this big list of metals; the AgNPs were given much attention due to its uniqueness in chemical, physical and biological properties. The chemical and physical methods generally utilize a large number of chemicals, require a huge space, and raise the environmental temperature, which sometimes becomes detrimental to the environment. On the contrary, the green synthesis or biological synthesis of AgNPs utilizes environmental benign substances, such as leaves, flowers, stem, bark, root, even microorganisms, etc [7,8].

The biological synthesis of AgNPs is a cost-effective, eco-friendly and relatively simple process and has a wide array of applications in the field of medicine like medical diagnosis and treatment as anticancer, antiviral, anti-inflammatory drugs, in the food industry for packaging of food materials, in air disinfection systems, in drinking water purification, metal ion recovery, analytical chemistry, etc [9–11]. The biosynthesis of AgNPs from bacteria entirely dependent on chemical and physical growth parameters, pH, temperature, growth medium composition, incubation period, growth in light and dark conditions, etc. There are few reports on the synthesis of AgNPs from bacteria, such as *Bacillus subtilis*, *Klebsiella pneumoniae*, *Corynebacterium* sp., *Aquaspirillum magnetotacticum, Desulfovibrio desulfuricans*, *Pseudomonas stutzeri*, *Lactobacillus* sp., and *Brevibacterium casei* [12].

Synthesis of AgNPs using bacteria is developing widely during recent years due to its immense applications in various fields of science. The extracellular synthesis of AgNPs from *Ralstonia* sp. strain NS-7 is used for the sustainable synthesis of polydispersed AgNPs. To the best of our knowledge and literature survey *Ralstonia* sp. has not been used for the synthesis of AgNPs. In this study, an attempt was made to green synthesis of AgNPs from *Ralstonia* sp. strain NS-7 and synthesized AgNPs was characterized by UV-vis, FTIR, XRD, AFM, SEM, HR-TEM, molecular taxonomical identification and phylogenetic study. Therefore, this study was aimed to synthesize AgNPs from the isolated bacterium from soil and the synthesized AgNPs was also used to assess their effect on biological systems.

2 Materials and Methods

2.1 Collection of soil samples, chemicals and microbial Cultures

Seven soil samples were collected from Tiwac field, Dharwad, Karnataka, India. The collected soil samples were immediately packed in sterile polythene bags and transported to the laboratory and stored at -20 $^{\circ}$C. The chemicals, such as Silver nitrate ($AgNO_3$, Sigma Aldrich), Sodium chloride (NaCl), Nystatin and Nutrient Agar medium were procured from Hi-media laboratories. Bacterial cultures like *Escherichia coli* (MTCC 40), *Enterococcus faecalis* (MTCC 6845), *Streptococcus pneumoniae* (MTCC 8874) and *Staphylococcus aureus* (MTCC 2825) were procured from IMTECH Chandigarh, India.

2.2 Isolation of soil bacteria

1g of soil sample was mixed in 10mL of 0.85% sterile NaCl solution. Serial dilution was carried out and 100μL of soil suspension was inoculated on Nutrient agar medium supplemented with Nystatin 25μg/L to inhibit fungal contamination. The plates were incubated at 37 $^{\circ}$C for 2 to 3 days for the proper growth of bacteria [13]. A total of 12 aerobic bacterial strains were isolated and among these 12 bacteria, only 1 bacterium was found gram-negative based on gram staining. The selected gram-negative bacterium was designated as strain NS-7.

2.3 Preparation of cell-free extract

Bacterial strain NS-7 was inoculated into 250 mL Erlenmeyer flask containing 100 mL sterile nutrient broth. The Bacterial culture was allowed to incubate on a rotating shaker set at 200 rpm at room temperature for 48h. After this, the bacterial culture was centrifuged at 12,000 rpm (REMI R-24) for 10 min. The culture supernatant was separated and used for the synthesis of AgNPs [14].

2.4 Biosynthesis of AgNPs

Aqueous solution of $AgNO_3$ (0.1mM/L) was prepared and used for synthesis of AgNPs. 80 ml of bacterial supernatant was added to 80 ml $AgNO_3$ solution. The pH was adjusted at 8.5 and kept in dark chamber for 3 days. The change in colour from

light yellow to brown represented the formation of AgNPs [15].

2.5 Characterization of AgNPs

2.5.1 UV-visible spectroscopy (UV-Vis)

The UV-visible absorption of AgNPs solution was measured by sampling of reaction mixture and absorption maxima at 200 nm to 700 nm by UV-visible spectrophotometer (Model-UV 9600 A UV/visible spectrophotometer series No: AU1701001) at USIC, Karnatak University, Dharwad. A spectrum of AgNPs was plotted with wavelength on X-axis and absorbance on Y-axis [16].

2.5.2 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy work was carried out to study possible biological functional groups involved in synthesis and stabilization of biological AgNPs. The FTIR measurements were performed using an R×1 instrument, Nicolet 6700 FTIR spectrophotometer, the FTIR spectra were scanned between 4000 and 400cm^{-1} at a resolution of 4cm^{-1} in the transmittance mode [17].

2.5.3 X-ray diffraction analysis (XRD)

The crystal structure and also the particle size were studied by Rigaku Miniflex 600 X-ray Diffractometry (XRD) analysis. The AgNPs powder was placed into the sample holder and a smooth surface was made through pressing. The XRD spectra were recorded between 30° and 80° by X-ray diffractometer equipped with CuKα filter (λ = 0.15418 nm), $2\theta/\theta$ scanning mode. The obtained diffractogram was compared to the standard JCPDS card No. 04-0783. The size of AgNPs was estimated using Scherrer's formula, d=0.89$\lambda/\beta\cos\theta$ [18].

2.5.4 Atomic Force Microscopy (AFM)

AFM work was carried for the study of morphology, size and distribution of AgNPs. Purified suspension was freeze dried to obtain dry powder. A thin film of the sample was prepared on a glass slide and it was allowed to air dry at room temperature, later the slides were scanned with the AFM instrument (Nano Surf AFM) [19].

2.5.5 Scanning Electron Microscope (SEM) and Energy Dispersive X-ray (EDX) analysis

SEM is a surface imaging method, fully capable of resolving different particle sizes, size distributions, nanomaterial shapes, and the surface morphology of the synthesized particles at the micro and nanoscales. The images were obtained using a SEM (JSM-IT 500LA). The purified suspension mixture of AgNPs was freeze-dried to obtain a dry powder and a very thin film of the AgNPs sample was prepared on the SEM stab and kept for gold coating, which was later scanned and analyzed with SEM. The chemical state and composition of the elements present in the AgNPs were also examined by EDX coupled with SEM [16].

2.5.6 High Resolution-Transmission Electron Microscope analysis (HR-TEM)

The diameter, size and morphology of the biological synthesized AgNPs can be analysed by using HR-TEM (Hitachi, Model: S-3400N). Double distilled water was used for dispersion of synthesized AgNPs sample. A drop of which was placed on a staining mat copper grid with carbon coat upwards. After 10 min air dried grid was removed and scanned in HR-TEM. The particle size and surface morphology of nanoparticles were evaluated using Image J 1.45s software [20].

2.6 Molecular taxonomical identification and phylogenetic study

The genomic DNA from fresh culture of the selected strain NS-7 was extracted. The PCR amplification of 16S rRNA gene was performed with universal primers. The conditions, which were used for the amplifications were initial denaturation of 25 cycles at 95 °C for 5 min, denaturation at 96 °C for 30 s, hybridization at 50 °C for 30 s and elongation at 60 °C for 1.30 min. The electrophoresed PCR products were on 1% agarose gel with 500 bp DNA ladder as the size reference. Purified PCR amplicons were sequenced (Sequence machine, applied biosystems, Sanger Sequencing 3500 Series, Genetic Analyzer). The partial 16S rRNA gene sequence of studied bacteria was analyzed with the nucleotide BLAST web portal in GenBank [21]. Phylogenetic relationship of this species was analyzed by the Neighbor-joining method with the other closely related bacterial species present in the Gene Bank using the software MEGA 4.0 by the method of Tamura et al., 2007 [22].

2.7 Antibacterial assay

The antibacterial activity of the biosynthesized AgNPs was tested against four pathogenic bacteria like *Escherichia coli*, *Enterococcus faecalis*, *Streptococcus pneumoniae* and *Staphylococcus aureus* by agar well diffusion technique. All these pathogens

were freshly cultured and swabbed separately on nutrient agar medium and later 6 mm wells were punched aseptically in the same medium. The working solution was prepared by making a suspension of 1 mg AgNPs in 1 mL sterile distilled water. From the working solution, 25 μl, 50 μl, 75 μl, and 100 μl of AgNPs suspension was pipetted into each well and incubated at 37 $^{\circ}$C overnight, the diameter of the inhibition zone around each well was measured in millimeter (mm) [(23)]. Streptomycin (10 μg/ml) solution was used as control.

2.8 Evaluation of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The antimicrobial efficacy of MIC and MBC to biologically synthesized AgNPs were studied using the standard broth dilution method (CLSI M07-A8). Determination of MIC against four pathogens, *Escherichia coli*, *Enterococcus faecalis*, *Streptococcus pneumoniae* and *Staphylococcus aureus*, was carried out on 96 well microtiter plate through broth dilution assay, whereas the MBC of AgNPs against the test bacterial pathogens was carried out on Muller-Hinton agar plates [(24)]. For MIC the bacterial inoculums concentration was adjusted to 0.10 at 625 nm (1×108 CFU/ ml, 0.5 McFarland's standard) from the stock solution and two-fold diluted AgNPs was seeded into each well, from column 12 to column 3 in the microtiter plate. The column 12 of microtiter contained the highest concentration of AgNPs, while column 3 contained the lowest concentration. Column 1 served as a negative control (only medium) and the column 2 served as a positive control (medium containing bacterial inoculums). Later; for each well of microtiter was added with 30μl of resazurin solution and the plates were allowed to grow for 24 h at 37°C. The results were read with reflected light, observing the presence or absence of macroscopic bacterial growth. The MIC value of AgNPs was calculated after 24 h of incubation. The MBC value of AgNPs was determined against the test bacteria and was performed by placing the suspension from the each well of microtiter plates into Muller-Hinton agar plates. The plates were incubated for 24 h at 37°C. The minimum concentration with no or negligible bacterial growth on Muller-Hinton agar plates was taken as MBC value.

3 Results

3.1 Biosynthesis of AgNPs

The Erlenmeyer flasks with the bacterial isolate strain NS-7 culture supernatant was pale yellow before the addition of $AgNO_3$ and this color was changed from pale yellow to brown at the end of the reaction with silver ions (Figure 1 A and B). The appearance of brown color in solution was a clear indication of the formation of AgNPs in the reaction mixture due to the reduction of Ag^+ ions to Ag^0 (elemental state) by the reducing agents in the cultural supernatant.

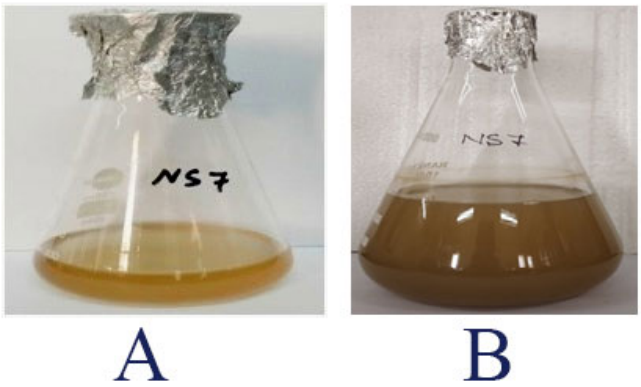

Fig 1. Synthesis of AgNPs from *Ralstonia* sp. strain NS-7: (A) Before adding $AgNO_3$, (B) after adding $AgNO_3$

3.2 Characterization of AgNPs

3.2.1 UV-visible spectrophotometric analysis of AgNPs

The UV absorption spectrum of the AgNPs synthesized by strain NS-7 was illustrated (Figure 2). The UV-visible spectroscopic observation of biological synthesized AgNPs from *Ralstonia* sp. strain NS-7 showed maximum absorption at 420 nm. The presence of more number of AgNPs results of the more reduction of silver ions, which might be due to the Surface Plasmon Resonance (SPR) property of AgNPs.

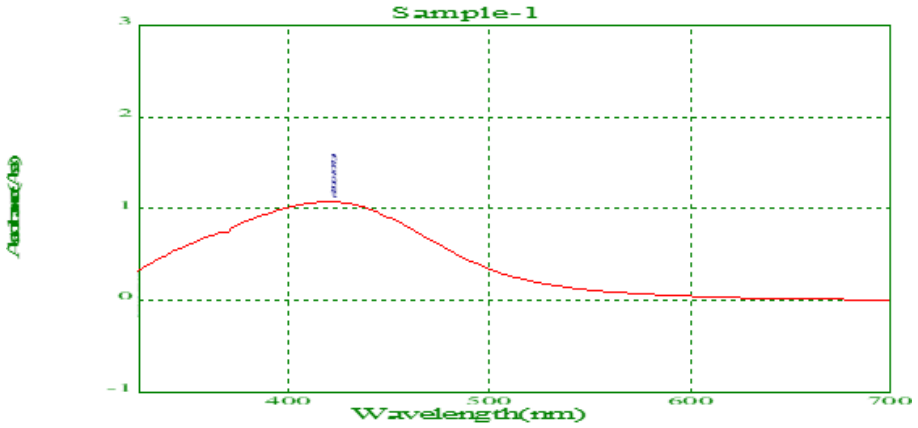

Fig 2. UV-Vis spectra of AgNPs synthesized by *Ralstonia* sp. strain NS-7

3.2.2 FTIR Analysis of AgNPs

The FTIR analysis of biological synthesized AgNPs was done to know the possible biological functional groups and bio-reducing molecules. The FTIR study revealed the ionising radiation (IR) bands at 668 cm^{-1}, 1027 cm^{-1}, 1233 cm^{-1}, 1278 cm^{-1}, 1383 cm^{-1}, 1453 cm^{-1}, 1544cm^{-1}, 1632 cm^{-1}, 1724 cm^{-1}, 2023 cm^{-1}, 2342 cm^{-1}, 2359 cm^{-1}, 2849 cm^{-1} and 3286 cm^{-1} wave numbers (Figure 3). The possible biological functional groups and bio-reducing molecules in the AgNPs from strain NS-7 was illustrated (Table 1). The biological functional groups like carboxylic acids, aldehydes, esters, aromatics, etc. were involved in the reduction, capping and stabilization of AgNPs.

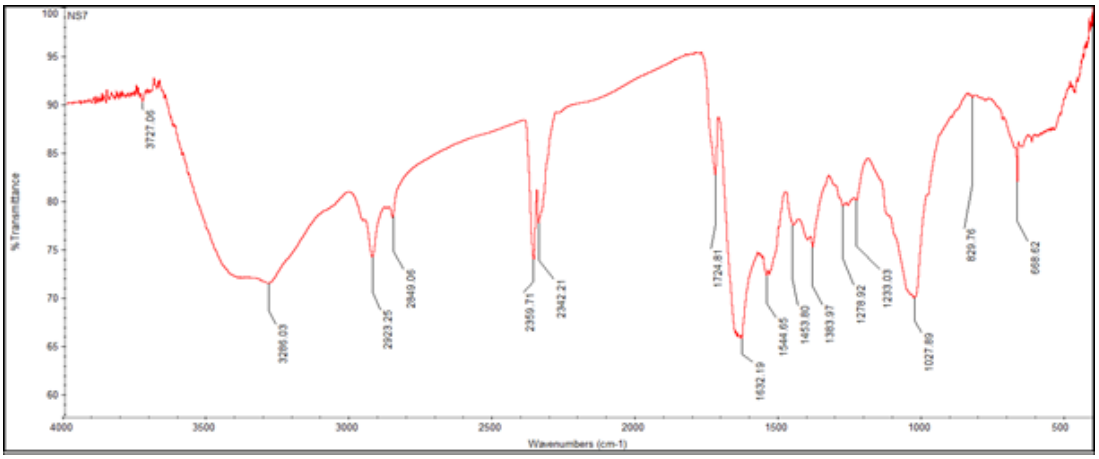

Fig 3. FTIR spectrum of AgNPs from the *Ralstonia* sp. strain NS-7

3.2.3 AFM analysis of AgNPs

AFM data revealed the particles were polydispersed and spherical and the size ranged from 18.17 nm to 78.12 nm in two and three-dimensional images of the nanoparticles (Figure 4 A and Figure 4 B).

Table 1. FTIR absorption peaks and their associated functional groups

Absorption peak (cm^{-1})	Functional groups
3286	O-H Stretching Carboxylic Acids
2023	N=C=S stretching Isothiocyanate
2849	C-H stretching Aldehydes
2359	P-H stretching Phosphine
2342	O=C=O stretching Carbon dioxide
1724	C=O stretch Esters
1632	C=C Stretching Mono-substituted alkenes
1544	-NH Stretching amides
1453	C-C stretching Aromatics
1383	-CH3 alkenes
1278	C-H Wag(-CH2X) Alkyl halides
1233	P-H bending Phosphine
1027	S=O stretching Sulfoxide
668	C-Br Stretching Alkyl halides

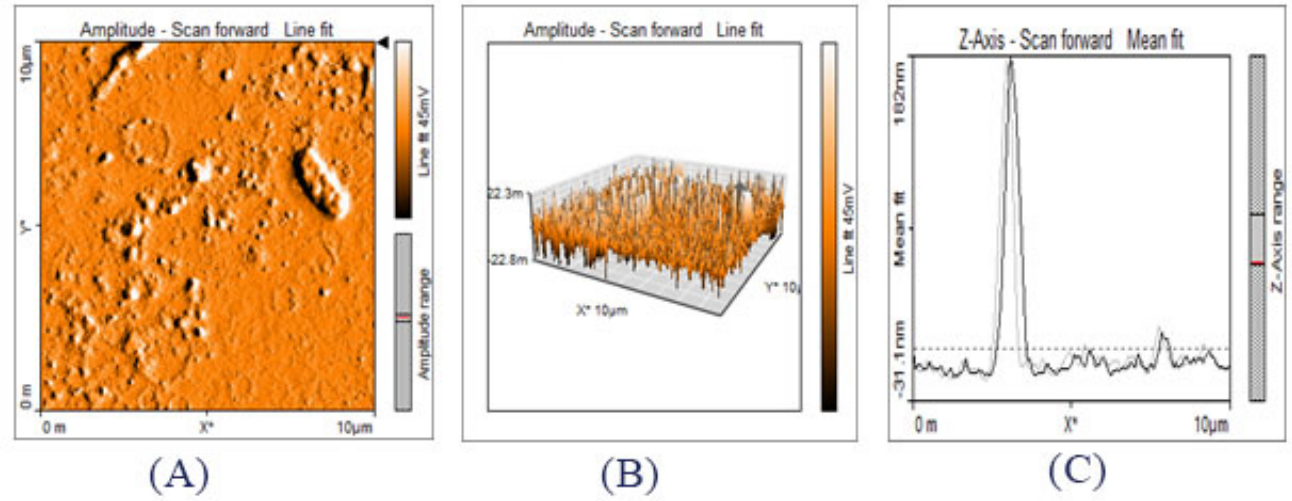

Fig 4. AFM analysis of synthesized AgNPs from the *Ralstonia* sp. strain NS-7: (A) The two-dimensional structure of AgNPs, (B) Three-dimensional structure of AgNPs, (C) The particle size distribution of AgNPs

The three-dimensional image showed the roughness and the homogeneity in the cluster formation of nanoparticles. The distance from each other was 39.06 nm (Figure 4 C).

3.2.4 SEM and EDX analysis of AgNPs

The 2μm resolution studies of biologically synthesized AgNPs with SEM revealed the particles were poly-dispersed and spherical in shape, having the size ranged from 18 nm to 42 nm. There was no agglomeration observed between the particles (Figure 5 A). The EDX analysis showed 54.61% presence of carbon in the sample along with 28.56% of oxygen, 2.01% of sodium, 0.35 of magnesium, and 3.51 of chlorine, 3.79% potassium and 7.17 % of silver (Figure 5B and Figure 5C). 7.17% of Ag metal in the sample indicates the presence of AgNPs.

3.2.5 HR-TEM analysis of AgNPs

The bio-reduced AgNPs were elucidated with the help of HR-TEM to determine the shape and average size of AgNPs. HR-TEM images of AgNPs derived from the strain NS-7 and were shown (Figure 6). The HR-TEM images of AgNPs at various

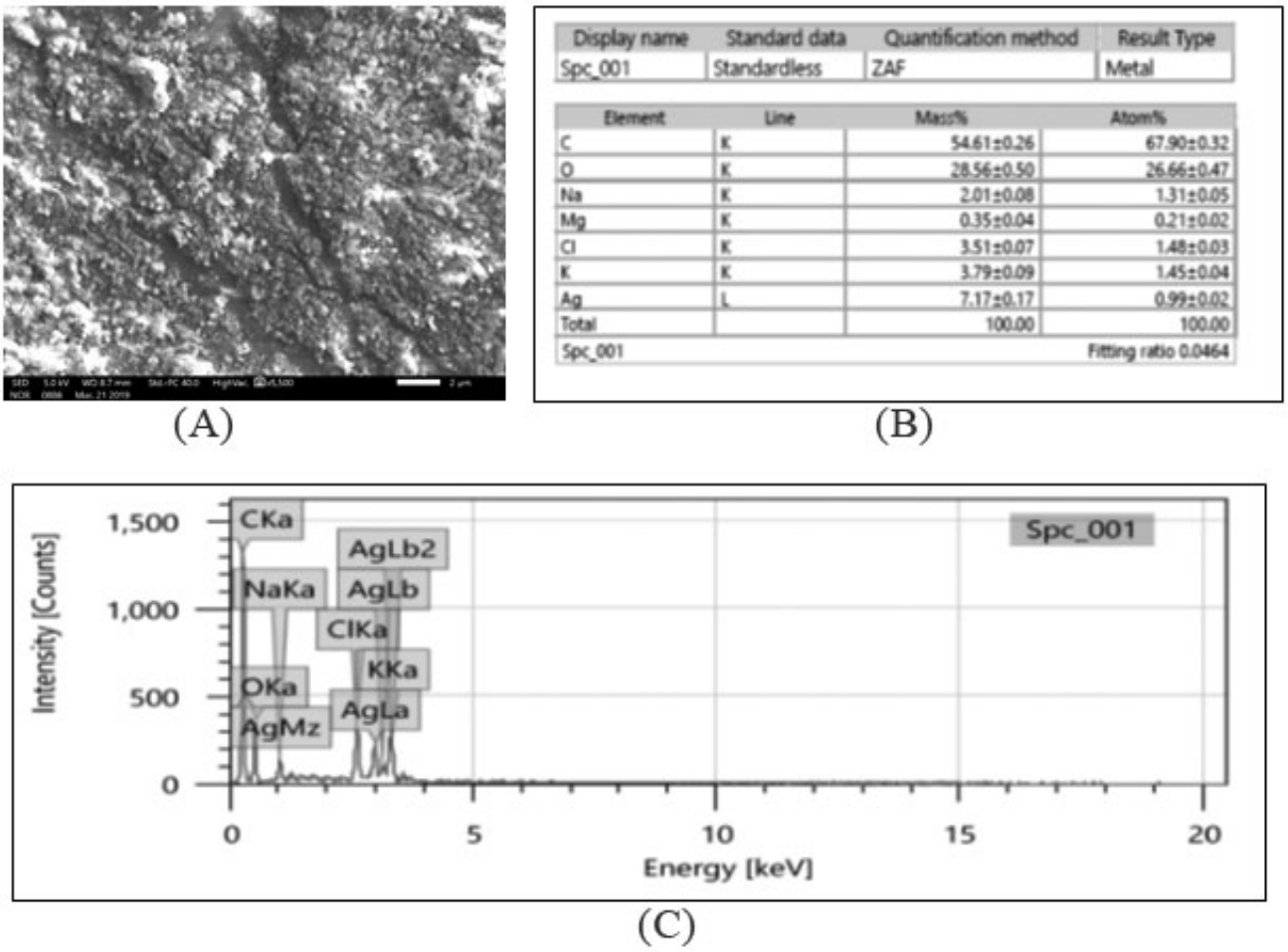

Fig 5. SEM with EDX for AgNPs, (A) Morphology of AgNPs from the *Ralstonia* sp. strain NS-7 studied by SEM, (B) EDX element composition in AgNPs, (C) EDX element composition graphs.

magnifications confirmed as spherical in shape. The diameter of AgNPs was ranged from 14.72 nm to 47.32 nm.

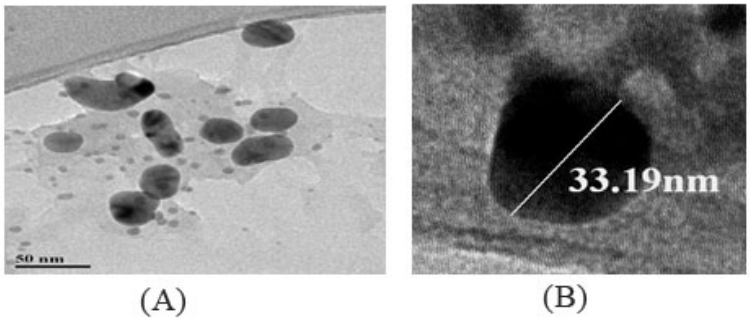

Fig 6. TEM micrograph of AgNPs from the *Ralstonia* sp. strain NS-7

3.2.6 XRD analysis of AgNPs

The X-ray diffraction (XRD) pattern of the prepared sample of AgNPs was examined. The XRD data and its analysis were shown (Figure 7). The diffractogram was compared with the standard powder diffraction card of a joint committee on the powder diffraction standard (JCPDS). The four distinct diffraction peaks at 38.1°, 46.2°, 64.5° and 77.3° in the experimental diffractogram was identified due to the presence of silver metal and corresponded to the values (111), (200), (220) and (311) planes of silver. The average particle size was calculated by using the Debye-Scherrer equation with full width at half maximum (FWHM) data used. The average particle size of AgNPs was estimated approximately 17.79 nm.

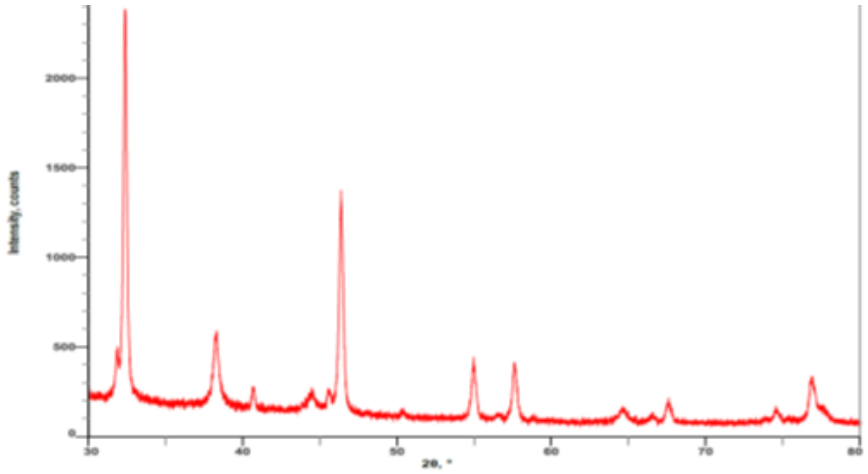

Fig 7. XRD spectrum of AgNPs from the *Ralstonia* sp. strain NS-7

3.3 Molecular taxonomical identification and phylogenetic study

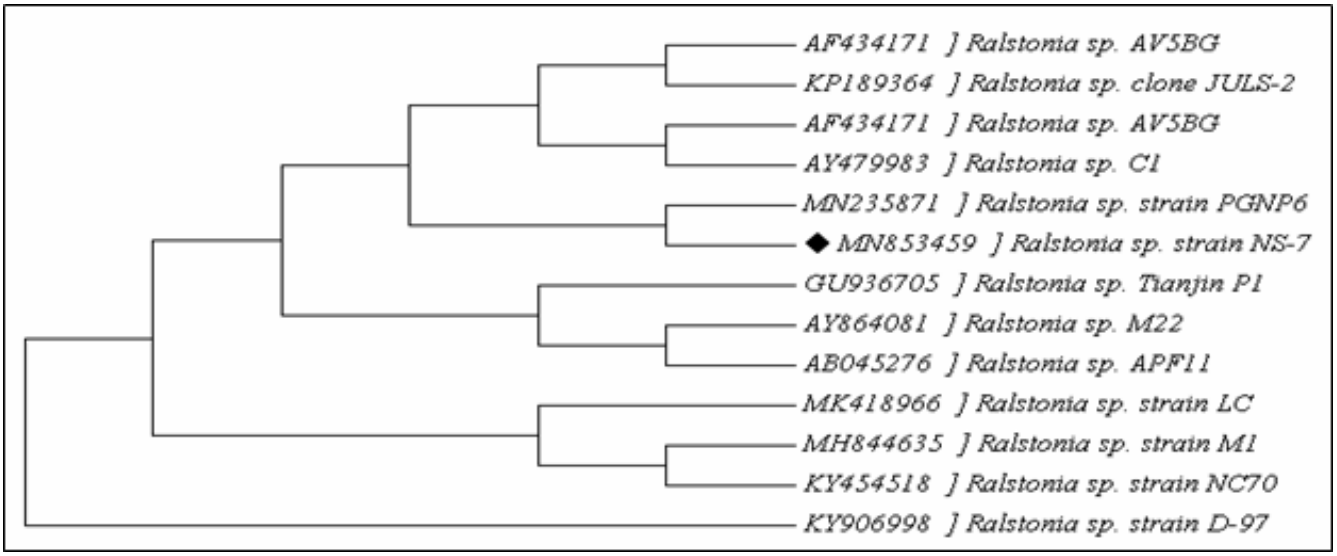

Fig 8. Phylogenetic relationships based on the 16S rRNA gene sequences

The molecular taxonomical identification of the strain NS-7 was done by 16S rRNA gene sequencing. The 16S rRNA gene sequence of strain NS-7 nucleotide sequence obtained was 772 base pair in length and was submitted to NCBI database with Accession No: MN853459. The sequence was initially characterized by BLAST analysis, which hit 100 homologous 16S rRNA genes from various species. Most homologies of the sequence were found with 16S rRNA genes of strain NS-7. The DNA

sequence was aligned and a phylogenetic tree was constructed by using MEGA-4.0 software (Figure 8). During the analysis of the phylogenetic tree, the *Ralstonia* sp. NS-7 revealed most sequence similarity with that of *Ralstonia* sp. PGNP6.

3.4 Antibacterial activity of synthesized AgNPs

The antimicrobial activity of biological synthesized AgNPs was studied on selected pathogenic bacterial strains like *Escherichia coli, Enterococcus faecalis*, *Streptococcus pneumoniae* and *Staphylococcus aureus* (Figure 9). The zone of inhibition formed at different concentrations of AgNPs against pathogenic bacteria was shown (Table 2). Among the bacterial pathogens, *E. faecalis* was found to be more sensitive and *E. coli* was the least sensitive. The biological synthesized AgNPs showed good antibacterial activity against gram-positive and gram-negative bacteria as compared to that of negative controls (strain NS-7 supernatant).

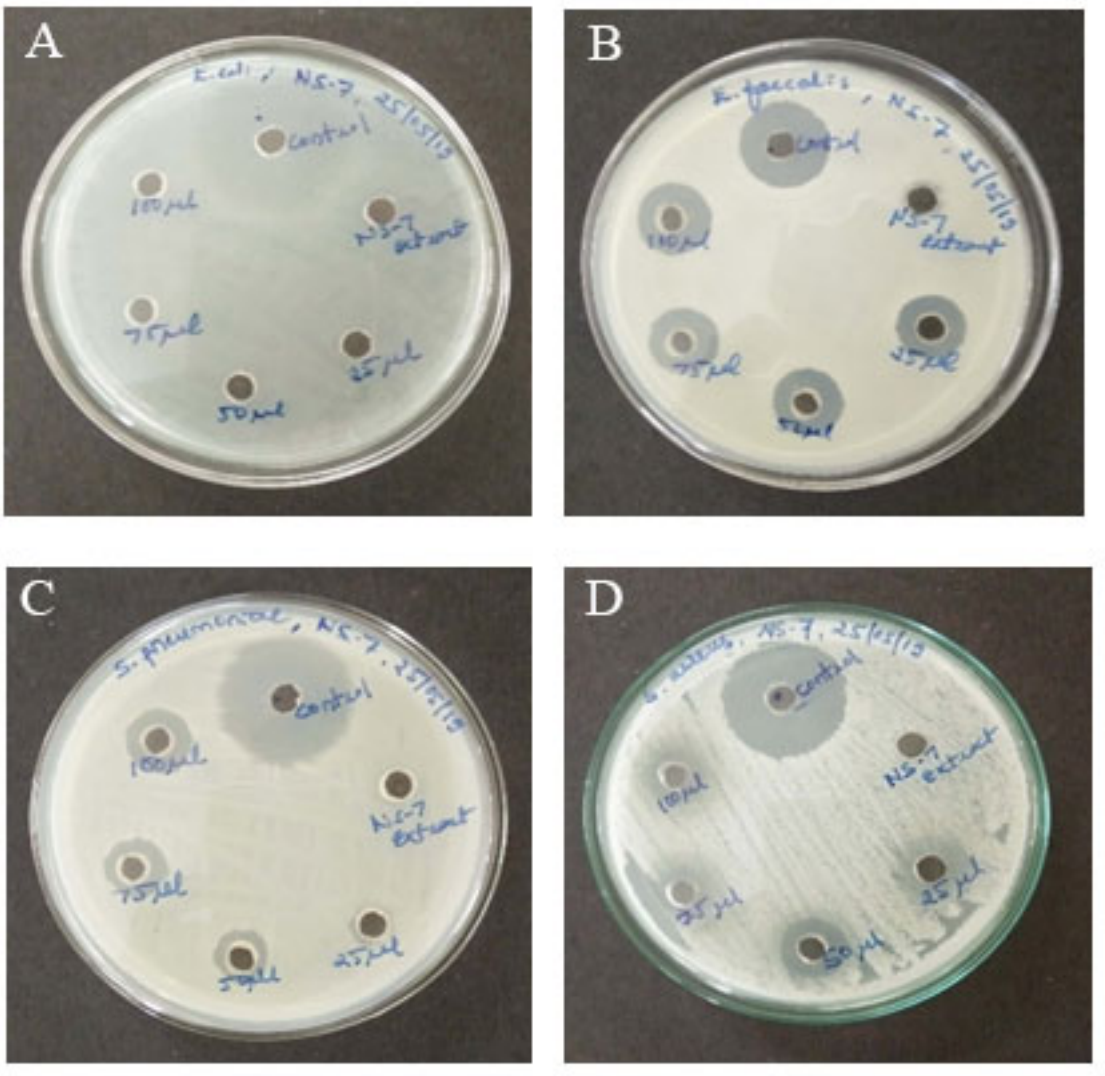

Fig 9. Antibacterial activity of biological synthesized AgNPs: (A) *Escherichia coli* (MTCC 40), (B) *Enterococcus faecalis* (MTCC 6845), (C) *Streptococcus pneumoniae* (MTCC 8874), (D) *Staphylococcus aureus* (MTTC 2825).

Table 2. Zone of inhibition formed at different concentrations of *Ralstonia* sp. strain NS-7 AgNPs against different microorganisms

Microorganisms	Zone of inhibition (mm) formed by AgNPs (1mg/mL)				Control, Streptomycin (50μl)(mm)	*Ralstonia* sp. strain NS-7 Supernatant (100μl) (mm)
	25μl	50μl	75μl	100μl		
E. coli (MTCC 40)	09	09	10	10	22	08
E. faecalis (MTCC 6845)	14	16	17	18	20	10
S. pneumoniae (MTCC 8874)	10	12	13	14	25	09
S. aureus (MTTC 2825)	13	15	16	17	25	08

3.5 MIC and MBC of AgNPs

The minimum bactericidal activity of biologically synthesized AgNPs was determined for all the four pathogenic bacteria. The MIC is defined as the minimum concentration of antibiotic substance required to kill or inhibit the growth of bacteria. The MBC value was determined at which there was no visible bacterial growth. Meanwhile, MIC value might be the subsequent lower to MBC, where less or no visible bacterial growth compared to the positive control. The MIC and MBC values were shown (Table 3). The MIC value of *Enterococcus faecalis* (MTCC 6845) was 9 μg/ml, whereas *Staphylococcus aureus* (MTCC 2825), *Streptococcus pneumoniae* (MTCC 8874) and *Escherichia coli* (MTCC 40) showed the MIC values 7 μg/ml, 8 μg/ml and 5 μg/ml respectively. The minimum bactericidal concentration obtained was 16 μg/ml for *Enterococcus faecalis*, 13 μg/ml for *Staphylococcus aureus*, 14 μg/ml for *Streptococcus pneumoniae* and 7 μg/ml for *Escherichia coli*.

Table 3. MIC(μg/ml) and MBC (μg/ml) values of AgNPs against bacterial pathogens

Sl. No.	Organisms	MIC (μg/ml)	MBC (μg/ml)
1	*E. faecalis* (MTCC 6845)	9	16
2	*S. aureus* (MTCC 2825)	7	13
3	*S. pneumoniae* (MTCC 8874)	8	14
4	*E. coli* (MTCC 40)	5	7

4 Discussion

AgNPs are emerging as a next-generation of applications in various fields of science and technology. This report presents for the first time data on the generation and synthesis of AgNPs using culture supernatants of *Ralstonia* sp. strain NS-7, which can be readily isolated from soil samples. The synthesis of AgNPs through the bacterial strain NS-7 showed good antibacterial activity against the selected pathogenic bacteria.

The bio-reduction of Ag^+ in the aqueous filtrate was monitored by periodic sampling of the reaction mixture at regular intervals by using UV-Vis. spectroscopy. The AgNPs exhibited light yellow to brown color and it was observed due to excitation of surface plasmon vibration of the metal nanoparticles [25]. The characteristic UV-Vis absorption band maximum present at 420 nm confirmed the presence of spherical or roughly spherical AgNPs. The spectrum showed a peak at 420 nm which was assigned to the SPR effect of silver indicating the particles were dispersed in the aqueous solution with no evidence for aggregation [26,27].

The FTIR analysis provides information about bio-reducing agents/functional groups present in the AgNPs, which is responsible for the transformation of $AgNO_3$ from simple inorganic to AgNPs. The existing free amino acids in proteins at 3286 cm^{-1} (Carboxylic groups) can bind to AgNPs and lead to the stabilization of AgNPs by the surface-bound free amino acids. The synthesized AgNPs have the strong ability to bind silver by forming carbonyl groups from the amino acid residues and peptides of protein. The proteins could form a coat covering the metal nanoparticles to prevent their agglomeration and aid in its stabilization in the medium. Similar results were reported by Fatima et al, 2017 [28].

The average particle size was calculated by the Debye-Scherrer equation, where full width at half maximum (FWHM) data was used. The average particle size estimated was approximately 17.79 nm. Similar results were reported by Gurunathan et al., 2009 [29]; Sachin et al., 2012 [30].The particles were spherical and some of the particles were agglomerated. Similar results were observed by previous studies in the surface topology of the synthesized SNPs as the primary method to monitor SNPs dissolution and agglomeration pattern [31] .

The SEM analysis clearly showed surface deposited AgNPs. The AgNPs were spherical, well defined and separated as much as possible. This may be due to the reduction in a liquid solution and some chelating action in the solution. Due to this silver particle nucleation is higher than the particle agglomeration [32–34]. SEM micrograph of AgNPs synthesized from *Ralstonia* sp. strain NS-7 taken at different magnifications showed the size of nanoparticles ranging from 18 nm to 42 nm. EDX analysis confirmed the presence of elemental silver and revealed a strong signal at approximately at 3 keV of the silver region. According to Mouxing et al., 2006 [34]; Ganesh and Suman, 2011 [35] reported that an EDX spectrum recorded in the spot-profile mode, the strong signals from the silver atoms and weaker signals from C, O, P and Na atoms were observed.

The HR-TEM images of AgNPs at various magnifications confirmed spherical shapes at different magnifications and sizes from 14.72 nm to 47.32 nm with the most apparent average size of 25.17 nm and approximately the synthesized AgNPs were homogeneous. The smallest AgNPs in strain NS-7 was 14.72 nm. Similar work was reported by Kalishwaralal et al., 2010 [5]. The data obtained from transmission electron micrograph showed distinct shapes and sizes of nanoparticles. The particles were spherical in shape with uniformly distributed without significant agglomeration.

The relatively high antibacterial activity could be attributed to the size and high surface area of the AgNPs, which enabled them to reach easily the nuclear content of bacteria [35]. It is suggested that AgNPs may attach to the surface of the bacterial cell membrane and release silver ions which may disrupt cell membrane permeability and bacterial DNA replication. The results observed about the bacterial cell membrane break down and release of the silver ions was reported by Gurunathan et al., 2009 [29]; Helene et al., 2002 [36]; Piuri et al., 1998 [37]. The present study showed that AgNPs synthesized by *Ralstonia* sp. have potential as antimicrobial compound against human pathogenic bacteria by showing the maximum zone of inhibition and may be used in the treatment of diseases caused by them.

The *Enterococcus faecalis* (MTCC 6845), *Staphylococcus aureus* (MTCC 2825), *Streptococcus pneumoniae* (MTCC 8874) and *Escherichia coli* (MTCC 40) showed the MICs values of 9 μg/ml, 7 μg/ml, 8 μg/ml and 5 μg/ml respectively. The MBCs obtained for *Enterococcus faecalis*, *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Escherichia coli* were 16μg/ml, 13 μg/ml, 14μg/ml and 7 μg/ml respectively. In a previous study, i*n vitro* Antimicrobial activity of green synthesized AgNPs against selected gram-negative foodborne pathogens showed the MICs and MBCs value of *Escherichia coli* were 7.8 μg/ml and 7.8 μg/ml respectively reported by Yuet et al., 2018 [38] .While MICs and MBCs values for *Staphylococcus aureus* were 12 μg/ml and 24 μg/ml respectively recorded in Antibacterial activity of AgNPs dispersion against MSSA and MRSA isolated from wounds in a tertiary care hospital by Mohammad et al., 2011 [39] . The MICs and MBCs for *Streptococcus pneumoniae* were 32 μg/ml and 64 μg/ml respectively reported by Wolfgang et al., 2010 [40] . On the other hand, AgNPs synthesized by Ramkumar et al., 2017, reported 62.5 μg/ml (MIC) and 250 μg/ml (MBC) values against *Enterococcus faecalis* [24]. The variation in the MICs and MBCs value might be due to the size and methodology employed for the synthesis of AgNPs.

5 Conclusion

The biologically synthesized AgNPs using *Ralstonia* sp. strain NS-7 showed broad-spectrum antimicrobial activity against gram-positive and gram-negative pathogens. It is an eco-friendly, green, non-toxic, economical and rapid method for the synthesis of AgNPs and therefore, could be used as an alternative source of antibiotics for the management of pathogenic and multi-drug resistant microorganisms.

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