



Characterization and antibacterial activity of silver nanoparticles synthesized by soil-dwelling *Bacillus thuringiensis* against drug-resistant bacteria

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Abstract

Alternative antibacterial options and nanotechnology have gained attention as a solution to the current antibiotic resistance crisis. The use of microorganisms to synthesize nanoparticles is applauded as a sustainable alternative for the production of metal-based nanoparticles. This research screened *Bacillus thuringiensis* (Bt) isolated from soils collected from different sites (farmland, cattle rangeland and metal recycling dumpsite) for their silver nanoparticles (AgNPs) synthesis potential, characterised the AgNPs and assessed their antibacterial activity against selected drug-resistant bacteria. The Bt index of the sampled sites ranged from 0.1 (farmland) to 0.5 (cattle rangeland). Out of six confirmed Bt isolates; MRS2¹ and CR2³ were selected for their AgNP synthesis potential based on their absorbance of AgNPs at 420 nm. Both isolates produced AgNPs which were anisotropic and irregular in shape. The surface plasmon resonance of silver occurred at 434.5 and 440 nm with distinct FT-IR peaks at 3379 and 1643 cm⁻¹. Apart from the multidrug-resistant *E. coli* (strain 1), the AgNPs from the two selected isolates exhibited antibacterial activity against *E. coli* (strain 2), *S. aureus* and *K. pneumoniae*. Antibacterial activity of the AgNPs was achieved at 25 µg/mL with MIC of 25–50 µg/mL and MBC of 75–100 µg/mL. Further studies are required to assess the effect of environmental factors on the properties of AgNPs synthesized, their mechanism of antibacterial activity and, synergistic effect of AgNP and antibiotics.

Keywords *Bacillus thuringiensis* · Silver nanoparticles · Drug-resistant bacteria · FT-IR · SEM · Antibacterial activity

Abbreviations

Bt	<i>Bacillus thuringiensis</i>
AgNO ₃	Silver Nitrate
AgNPs	Silver Nanoparticles
NP	Nanoparticles
LB	Luria Bertani
MHA	Muller-Hinton Agar
MRS	Metal Recycling Site
CR	Cattle Rangeland
FTIR	Fourier-Transform Infrared Spectroscopy
SEM	Scanning Electron Microscopy
XRE	Transcriptional Regulator
MIC	Minimum Inhibitory Concentration
MBC	Minimum Bactericidal Concentration

MAR	Multiple Antibiotic Resistance
MDROs	Multi Drug Resistant Organisms
MRSA	Methicillin-Resistant <i>Staphylococcus aureus</i>
VRSA	<i>Vancomycin</i> -resistant <i>Staphylococcus aureus</i>
TNF	Tumour Necrosis Factor

Introduction

The global public health crisis caused by antimicrobial resistance emphasize the need for the development of alternative strategies (Lee et al. 2019). These alternatives have become crucial as antibiotics pipeline dry out and, the efficiency of existing antibiotics diminish (Gao and Zhang 2021). Nanotechnology is developing rapidly with applications in various fields including biotechnology and medical research (Thakkar et al. 2010; Herlekar et al. 2014; Phanjom and Ahmed 2015; Yurtluk et al. 2018). There are growing interests in the potential of nanoparticles in limiting the problem of antimicrobial resistance. Nanoparticles (NPs)

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have gained attention as a result of their unique physico-chemical properties and broad-spectrum antibacterial activity (McNeilly et al. 2021).

Nanoparticles (NPs) proffer a promising therapeutic alternative to combating multidrug-resistant organisms (MDROs) due to their physical and chemical properties (Lee et al. 2019). The mechanisms of action include cell membrane and cell wall disruption, oxidative stress by reactive oxygen species generation and lipid peroxidation. However, the antibacterial properties of NPs may be affected by physicochemical properties such as size, shape, chemical modification, and environmental factors during preparation (Lee et al. 2019). Examples of metallic NPs and their oxides already applied as active antimicrobial agents include silver, gold, copper, zinc, aluminium, iron, titanium dioxide, and silica NPs of which silver is known to be most toxic to bacteria (Lee et al. 2019). Previous studies showed that silver nanoparticles with a size range of 10–100 nm exhibit potent bactericidal activity against bacteria including multidrug resistant bacteria such as *Pseudomonas aeruginosa*, ampicillin-resistant *Escherichia coli*, erythromycin-resistant *Streptococcus pyogenes*, methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Staphylococcus aureus* (VRSA). AgNPs may also exhibit other activities including fungicidal activity against *Aspergillus*, *Candida* and *Saccharomyces*, and antiviral activity against HIV-1, suppressing tumour necrosis factor (TNF), interleukin (IL)-12 and IL-1b and inducing apoptosis of inflammatory cells (Rai et al. 2012). NPs have also been conjugated with antibiotics resulting in synergistic effects against bacteria including MDROs and biofilm formation (Gupta et al. 2017; Ruddaraju et al. 2020). NP coatings can also act as NP-based antibiotic delivery systems in implantable devices and dental materials (McNeilly et al. 2021).

Metallic Nanoparticles may be synthesized by various physical and chemical methods (de Mel et al. 2012; Tsuji et al. 2012; Rani et al. 2017). Although, these methods provide higher productivity and better size control of synthesized NPs, their limitations include high cost and energy requirements, and use of toxic chemicals which generate toxic wastes (Fariq et al. 2017). On the other hand, biosynthetic methods involving microorganisms, are advantageous in terms of rapid synthesis, eco-friendliness and less toxicity. Bacteria are commonly used as their extraordinary ability of reducing heavy metal ions have been (Mustapha et al. 2022). Biosynthesis using bacteria can occur either intra- or extra-cellularly. Extracellularly, reduction of metal ions for NPs synthesis occurs in the presence of microbial enzymes and proteins, bacterial or fungal cell wall components, or organic molecules present in the culture medium (Siddiqi et al. 2018).

Therefore, in this present work aimed at synthesizing extracellular AgNPs by *Bacillus thuringiensis* isolated from

soil samples from different sites in Kaduna state, Nigeria, followed by characterizing the AgNPs and investigating their antibacterial activity against some drug resistant bacteria.

Methods

Bt isolation and characterization

Soil samples were collected from three sites namely, Pepper farmland, Cow rangeland and Metal recycling site in Zaria, Kaduna State, Nigeria. The soil samples were serially diluted in sterile distilled water, then 2 mL of the broth culture was added to 20 mL of Luria Bertani (LB) (Tryptone 10 g/L, yeast extract 5 g/L, NaCl 5 g/L) broth buffered with 0.25 M sodium acetate (pH 6.8) in 50 mL Erlenmeyer flask and was incubated for 4 h at 28°C on a rotatory shaker (B. Bran scientific and instrument company, England) at 250 rpm (Travers et al. 1987; Rampersad and Ammons 2005). Thereafter, 1 mL of the aliquot were plated onto sterile LB agar and incubated at 28°C for 48 h. The resulting colonies were subcultured on T3 agar (Tryptone 3 g/L, yeast extract 1.5 g/L, Peptone 2 g/L, MnCl₂ 0.005 g/L, Sodium phosphate 0.05 M and Agar 15 g/L). The characteristic cultural and biochemical properties of the suspected Bt strains were observed (Cowan and Steel 2003; Bergey 2004) following Gram staining and spore staining procedures. Biochemical tests conducted include: motility, catalase, oxidase, citrate utilization, Methyl red-Voges Proskauer, casein hydrolysis, and arginine hydrolysis. *Bacillus thuringiensis* isolation index (Bt Index) was calculated by dividing the number of Bt isolates by the total number of Bacillus-like colonies obtained. Presumptive Bt isolates were subjected to DNA extraction using Qiagen DNA easy kit (Jiangsu Mole Bioscience Co., Ltd, China) and polymerase chain reaction to detect transcriptional regulator (XRE) with the XRE specific primers (Forward “AAGATATTGCAAGCGGTAAGAT” and Reverse “GTTTTGTTTCAGCATTCAGTA” using PCR conditions described by Wei et al. (2019). Amplicons were visualized by 1.5% agarose gel electrophoresis.

Screening of Bt isolates for biosynthesis of AgNPs

The method described by Agrawal and Kulkarni (2017) was used to screen and select the most efficient Bt isolate for the bio-production. The Bt isolates were subcultured on LB agar supplemented with 1mM concentration of aqueous silver nitrate (AgNO₃) solution at 28°C for the screening. The isolates were further screened for optimal biosynthesis of AgNPs by methods described by Kumar et al. (2018). The isolates which produced AgNPs with the highest absorbance when observed at 420 nm in a spectrophotometer were considered the most efficient in the biosynthesis of AgNPs.

Extracellular biosynthesis and characterization of AgNPs from the selected Bt strain

The biosynthesis of AgNPs was carried out as described by Ojo et al. (2016) with slight modification. One mL of standardized inoculum of Bt prepared according to McFarland's scale 0.5 (1.5×10^8 cells/mL) was inoculated in 30 mL LB broth and incubated for 24 h at 37 °C. The culture was centrifuged at 4000 rpm for 15 min and the cell-free supernatant was used for the extracellular biosynthesis of AgNPs. Thereafter, 30 mL of AgNO₃ (1 mM) was mixed carefully with 1 mL of cell-free supernatant in 250 mL conical flask for the reduction of silver nitrate at 28 °C and static conditions for 24 h. The control without the AgNO₃ (cell-free supernatant) was held at the same conditions and change in colour was observed visually (Lateef et al. 2014).

Following the extracellular synthesis, the AgNPs solution was scanned at 250–800 nm using UV-Visible Spectrophotometer (UV-Vis) (Jenway-6405) to verify the reduction of Ag⁺ ions. 5 mL AgNPs solution was centrifuged at 4000 rpm for 20 min, and the residue scanned between 700 and 4000 cm⁻¹ using Fourier Transform-Infrared (FT-IR) spectroscopy (Shimadzu FTIR-spectrophotometer) (Lateef et al. 2014) at the National Research Institute of Chemical Technology (NARICT), Zaria, Nigeria. This was carried out to characterize the interaction between biomolecules (proteins) and AgNPs, and quantify secondary structure in metal nanoparticle–protein interaction (Elamawi et al. 2018). SEM analysis was used to determine the morphology (in terms of size and shape) of AgNPs synthesized by the isolates. 5 mL AgNPs was centrifuged at 4,000 rpm for 15 min, and the supernatant was discarded while the pellets were used for the SEM analysis. The pellet was mixed properly and carefully placed on a stud. The analysis was done using Phenom ProX SEM machine (Phenom World Eindhoven, The Netherlands) available at the National Steel Raw Materials Exploration Agency (NSRMEA), Federal Ministry of Mine and Steel Development, Kaduna, Nigeria. The image of AgNPs was obtained using a desktop computer connected to the scanning electron microscope.

Antibacterial activity

Agar well diffusion method was used to assess the antibacterial activity of silver nanoparticles synthesized against four selected multidrug resistant pathogenic bacteria namely; *Escherichia coli* (test pathogen 1), *Escherichia coli* (test pathogen 2), *Staphylococcus aureus* and *Klebsiella pneumoniae*. Standardized suspension of each selected test organism (0.5 McFarland's standard; 0.5×10^6 CFU/mL) was swabbed uniformly onto sterile Muller-Hinton Agar (MHA) plates using sterile cotton swabs. Wells of 9 mm diameter were bored into the agar medium using gel puncture. An aliquot of 100 µL of the silver nanoparticles at different concentrations (100 µg/mL, 75 µg/mL, 50 µg/mL and 25 µg/mL) was added into each well. After incubation at 37 °C for 24 h, zones of inhibition were measured. A cell-free supernatant of Bt culture and ciprofloxacin (antibiotics) were used as negative and positive controls for antimicrobial activity respectively.

Results

Isolation and characterization of Bt isolates and screening for AgNPs biosynthesis

A total of 30 isolates with *Bacillus*-like characteristics (creamy white, circular, dry, flat elevation and with wavy margin) were observed on T3 agar. The Bt- index ranged from 0.1 to 0.5 (Table 1). A total of ten Bt isolates were identified based on cultural, microscopic (presence of rod-shaped cells, oval shape of spores, and subterminal spore position) and biochemical characterizations (motile, positive for catalase, citrate, MR, VP and starch hydrolysis and negative for indole). XRE gene was detected by PCR (246 bp) in six out of the ten isolates (Fig. 1) and two isolates were more efficient in AgNPs production with the highest absorbance reading at 420 nm (Table 2). These were used for the extracellular biosynthesis of the AgNPs. The extracellular biosynthesis of AgNPs by Bt isolates MRS2¹ and CR2³ was confirmed by visual observation with the appearance of colour change of supernatant from whitish to dark brown in the reaction mixture within 24 h while the control (without Bt isolates) showed no colour change.

Table 1 Distribution and Bt index of *Bacillus thuringiensis* isolated from selected soil sites in Zaria

Source of soil samples	Number of Bacillus-like isolates	Number of Bt isolates	^a Bt index	Frequency of distribution of Bt (%)
Farmland	10	1	0.1	10
Cattle ranch	10	5	0.5	50
Metal recycling dumpsite	10	4	0.4	40
Total	30	10	1.0	100

^aBt Index: *Bacillus thuringiensis* isolation index was calculated by dividing the number of Bt isolates by the total number of Bacillus-like colonies obtained

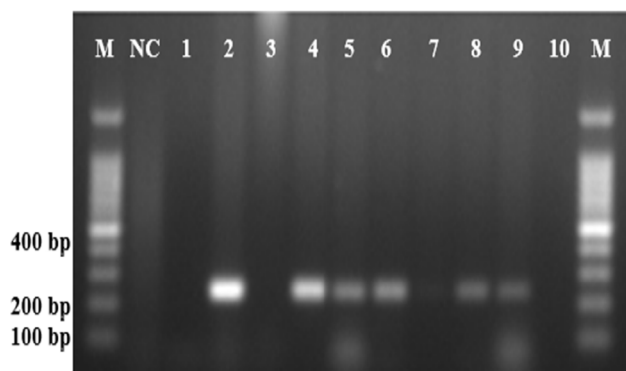


Fig. 1 Amplicons of XRE gene (246 bp) from Bt isolated from soil samples. Key: Lane M: 100 bp DNA ladder marker (Biolabs); Lane NC: Negative control; Lane 1, 7, 8, 9 and 10: Bt isolated from Cattle rangeland; Lane 2, 4, 5 and 6: Bt isolated from Metal recycling site and Lane 3: Bt isolated from Pepper farmland

Table 2 Absorbance of silver nanoparticles produced by the Bt isolates at 420 nm

Bt Isolate Code	Absorbance
CR2 ³	0.811
MRS1 ³	0.303
CR2 ²	0.173
MRS2 ¹	0.879
MRS2 ²	0.481
MRS2 ³	0.200

Key: CR- Cattle rangeland; MRS- Metal Recycling Site

Characterisation of synthesized AgNPs by UV-Vis, FT-IR spectroscopy and SEM analysis

The surface plasmon resonance (SPR) of silver occurred at 434.5 and 440 nm, which was attributed to the SPR band of AgNPs (Fig. 2). Figure 3 shows the FT-IR spectra analysis of the AgNPs produced by the isolates. The FT-IR absorption spectra showed distinct strong peaks at 3379, and 1643 cm^{-1} . Other minor peaks at 3942, 3865, 3796, 2407, 2137, 1265 and 1087 cm^{-1} were obtained. The SEM micrograph obtained as shown in Fig. 4 shows that the particles were majorly irregular in shape with size of 748 nm.

Antibacterial activity of AgNPs against selected drug resistant bacteria

The MAR index of the test organisms ranged from 0.1 to 0.5 for *S. aureus* and *E. coli* (test strain 1) respectively (Table 3). Also based on the antibiotic resistance pattern of the test organisms, only *E. coli* (test strain 1) was found to be multidrug resistant. Multidrug resistance refers to the resistance to three or more classes of antibiotics (Sweeney et al. 2018). AgNPs produced from both *B. thuringiensis*

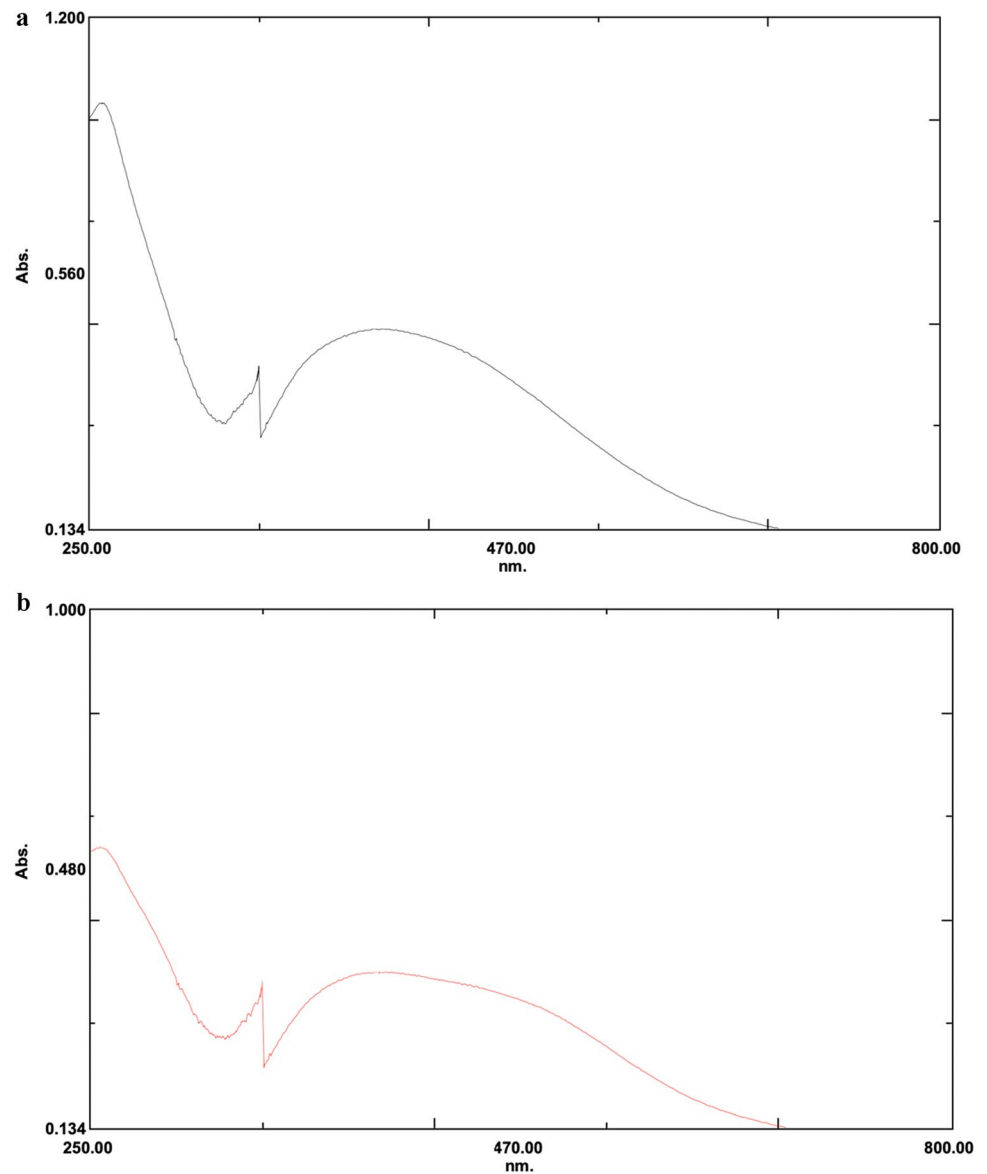
isolates CR2³ and MRS2¹ did not exhibit antimicrobial activity (25–100 $\mu\text{g}/\text{mL}$) against the MDR resistant isolate; *E. coli* (strain 1). However, AgNPs from *B. thuringiensis* CR2³ and MRS2¹ exhibited antibacterial activity against the other drug resistant bacterial isolates with zones of inhibition of 13–19 mm (Table 4) and 11–22 mm (Table 5) respectively. Also, the AgNPs exhibited a MIC of 50 $\mu\text{g}/\text{mL}$ and 25–50 $\mu\text{g}/\text{mL}$, while the MBC was 100 $\mu\text{g}/\text{mL}$ and 75–100 $\mu\text{g}/\text{mL}$ respectively (Table 6).

Discussion

This study isolated *Bacillus thuringiensis* from different sites and assessed their silver nanoparticle production potential. The AgNPs were also characterised by FT-IR and SEM analysis and their antibacterial activity assessed against some drug resistant pathogens. The number of Bt isolates varied with the sampled sites with a Bt index ranging from 0.1 (farmland) to 0.5 (cattle ranch). The XRE gene was detected in six out of ten *Bacillus thuringiensis*. Following the preliminary screening for AgNPs synthesis, two isolates; CR2³ and MRS2¹ were identified with AgNP production potential based on their absorbance readings of 0.811 and 0.879 respectively. Further confirmation was achieved by UV spectrophotometry which showed peaks at wavelength of 434.5 and 440 nm for isolate CR2³ and MRS2¹ respectively. The SEM image showed that the particles were predominantly irregular and anisotropic in shape of 748 nm. The test pathogens exhibited different antibiotic resistance pattern with a MAR index of 0.1–0.5 with *E. coli* (strain 1) exhibiting multidrug resistance. AgNPs exhibited antibacterial activity against *E. coli* (strain 2), *Klebsiella pneumoniae*, and *Staphylococcus aureus* but not against the MDR *E. coli* (strain 1).

The characterization of ten Bt isolates from soil agrees with that of Eswarapriya et al. (2010) that reported that the strains of Bt, were positive for catalase production, citrate utilization and starch hydrolysis. The amplification of XRE gene in six out of ten isolates is supported by the report of Wei et al. (2019) that there is 97.3% accuracy when using XRE gene to distinguish *B. cereus* and Bt as this is the transcriptional regulator which regulate the major type of crystal production. The UV–visible Spectroscopy was used to confirm the synthesis of nanoparticles to detect surface plasmon resonance (SPR). AgNPs provide the SPR band because the conduction band and valence band of AgNPs lie close to each other, making photons to move freely and vibrate each electron. When the collective oscillation frequency of electrons becomes equal to the incoming wave frequency, then strong absorption takes place, which leads to the production of surface plasmon resonance (Fozia et al. 2022). The findings on the absorbance of AgNPs are in correlation with the experimental findings of Ojo et al.

Fig. 2 **a** UV-Vis absorption spectrum showing characteristic peak at 434.50 nm of AgNPs produced by Bt isolate MRS2¹. **b** UV-Vis absorption spectrum showing characteristic peak at 434.50 nm of AgNPs produced by Bt isolate CR2³



(2016) and Lateef et al. (2014). Furthermore, the absorption spectrum of AgNPs observed supports the findings of other authors (Jain et al. 2010; Prakash et al. 2011; Dhoondia and Chakraborty 2012; Kumar et al. 2018) that reported peaks of UV absorption spectra of AgNPs in the range 391–440 nm which is the characteristic peak for AgNPs. The irregular and anisotropic shapes conform to the types of NPs shapes reported by Tarannum et al. (2019) and it also supports the result of the UV-visible scan analysis that showed a broad peak indicating the presence of more than one shape. With respect to the size of the AgNPs (748 nm), several authors have reported a range of sizes for AgNPs. Murthy et al. (2014) reported sizes of AgNPs facilitated by Bt ranging from 32 to 1106 nm. Saravana et al. (2015) also reported AgNPs size of 198–595 nm which was mediated by *Streptomyces* sp. However, some studies have reported

smaller sizes such as; Kumar et al. (2018) reported AgNPs sizes of 5–15 nm while Krishna et al. (2017) have reported AgNPs sizes ranging from 15 to 25 nm mediated by white rot fungi. The antibacterial activity of AgNPs against the test organisms irrespective of their Gram reaction, is supported by the reports of Feng et al. (2000), Sondi and Salopek-Sondi (2007), Ravishankar, and Jamuna (2011). Our finding of zones of inhibition at a lower concentration contradicts Elbeshehy et al. (2016) who stated that smaller sized NPs are better antimicrobial agent as they can easily penetrate the cell wall considering the fact that the AgNPs produced by the cited authors were smaller in size. Although, Yurtluk et al. (2018) tested the antibacterial potential of the silver nanoparticles synthesized by *Bacillus* sp SBT8 on *S. aureus*, and *E. coli* 0157: H7 and observed zones of inhibition of 11 mm and 8 mm respectively at

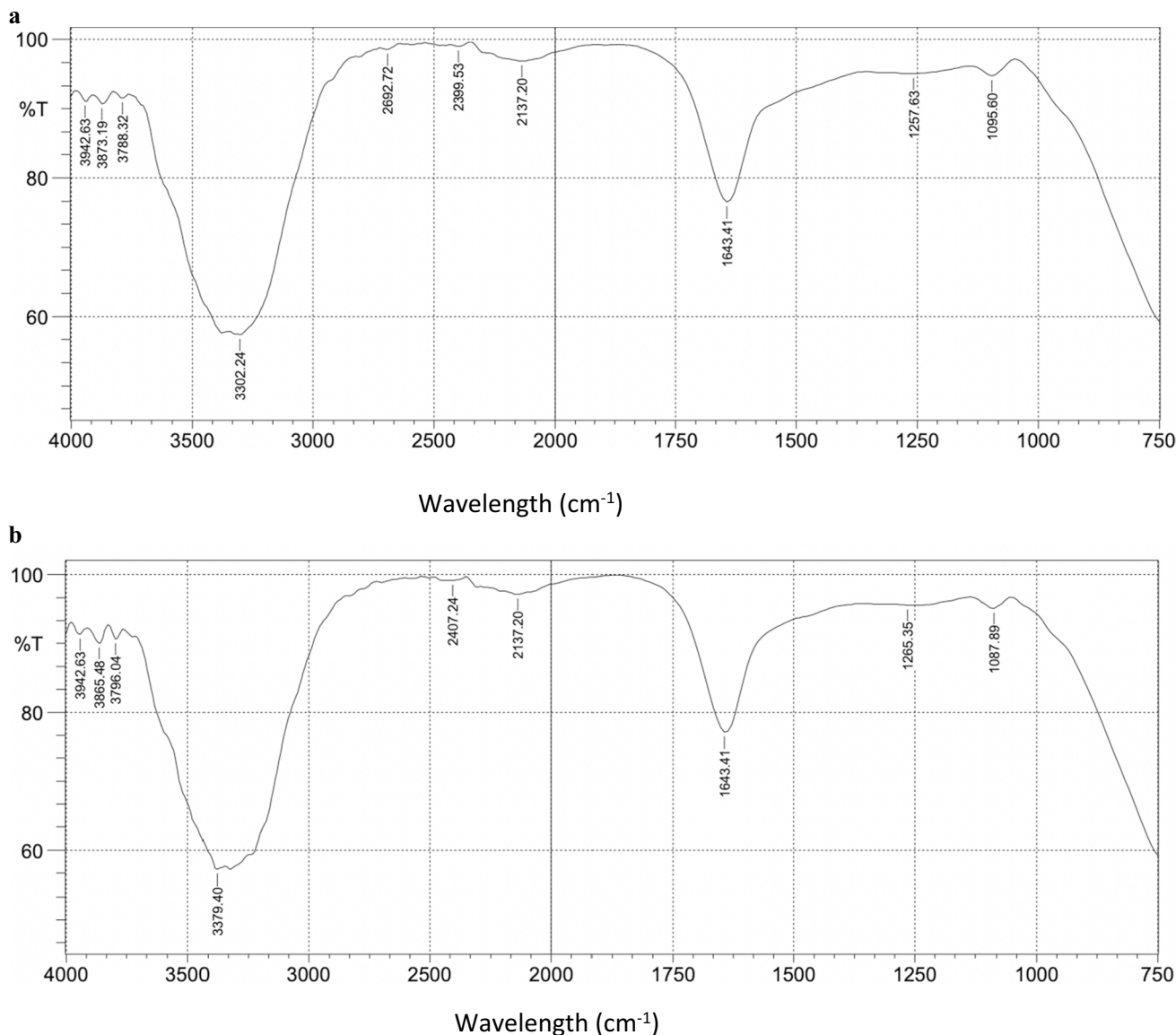


Fig. 3 **a** FT-IR spectrum of AgNPs facilitated by Bt isolate MRS2¹. **b** FT-IR spectrum of AgNPs facilitated by Bt isolate CR2³

10 µg /mL of synthesized AgNPs. Lateef et al. (2014) studied the antibacterial effect of AgNPs produced by using *Bacillus safensis* LAU 13 on some clinical *E. coli* strains and they reported 8.6 to 12.5 mm zone of inhibition at 150 µg /mL. In another study, the inhibitory effect of AgNPs were tested on *E. coli*, *Pseudomonas aeruginosa*, and *S. aureus*, and 60–100 µg/mL concentrations inhibited these bacteria (Lateef et al. 2015; Alsamhary 2020) also reported MIC at higher concentration of 300 µg /mL for *Klebsiella pneumoniae* as against MIC of 25 µg /mL (MRS2¹) and 50 µg /mL (CR2³) observed in this study. The lack of antibacterial activity of both AgNPs against MDR *E. coli* (strain 1) compared to the other test bacteria may be supported by recent studies raising concerns about

the emergence of silver-resistant bacteria apart from the previously known resistance to cationic silver (Ag⁺) (Hosny et al. 2019; Valentin et al. 2020; McNeilly et al. 2021).

Although Bt is known to be a cosmopolitan environmental bacterium, the higher Bt index in cattle ranch site compared to the other sites may be attributed to high organic matter in the soils which favours the proliferation of Bt. Bt exhibit a saprophytic lifestyle by using decaying organic matter or roots exudates as a rich source of nutrients hence, also described as copiotrophic (Argôlo-Filho and Loguercio 2014). As absorbance is proportional to concentration, the darker the colour of the AgNPs formed, the higher the absorbance (Lateef et al. 2014).

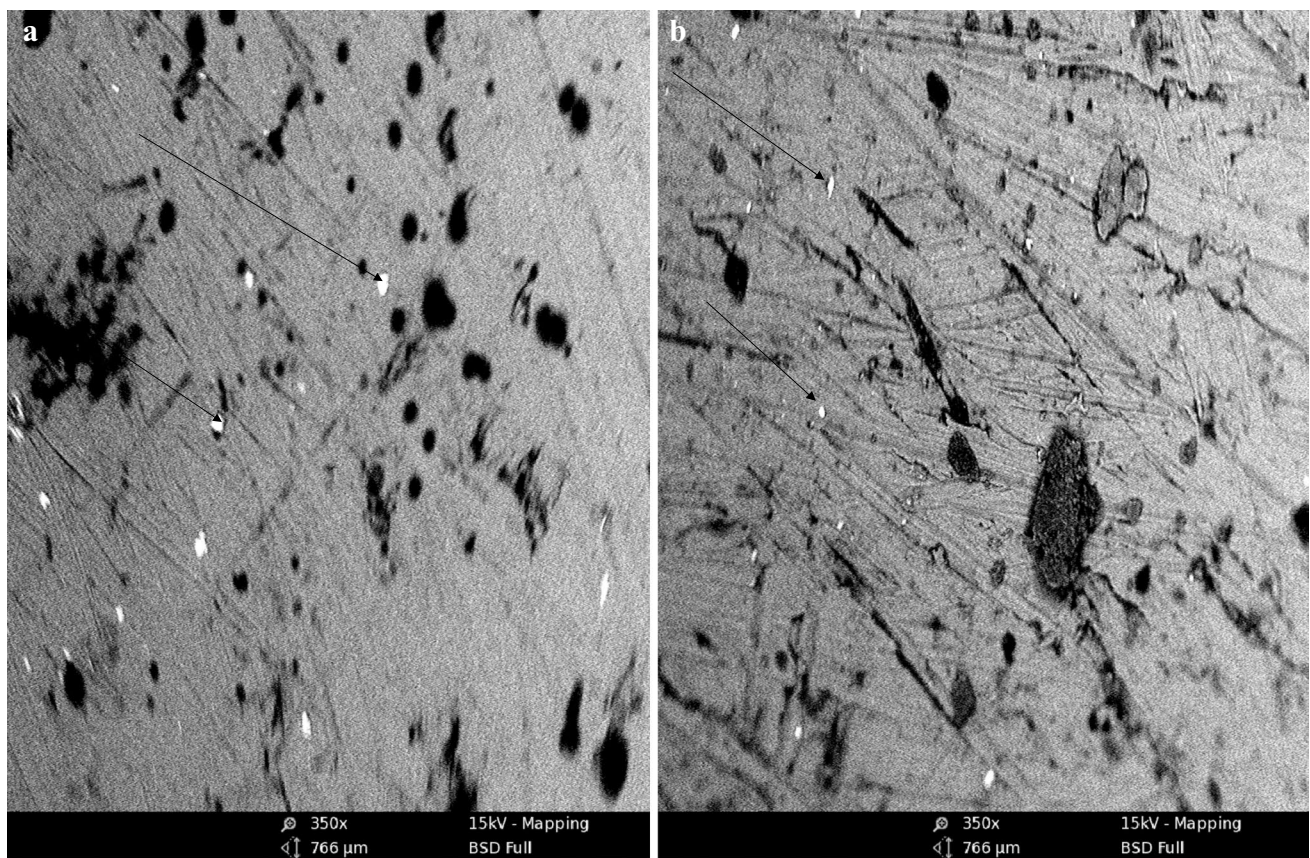


Fig. 4 **a** SEM image of AgNPs facilitated Bt isolate MRS2¹. Arrows point towards AgNPs biosynthesized by Bt isolates. **b** SEM image of AgNPs facilitated Bt isolate CR2³. Arrows point towards AgNPs biosynthesized by Bt isolates

Table 3 Antibiotic resistance pattern and MAR index of test organisms used for antibacterial assay

Test organisms	Source	Resistance pattern	MAR index
<i>E. coli</i> (strain 1)	Urine	AMC, TE, CPX, CFT and AMP	0.5
<i>E. coli</i> (strain 2)	Urine	AMC, TE and AMP	0.3
<i>S. aureus</i>	Chicken	AMP	0.1
<i>K. pneumoniae</i>	Urine	SP, AMP and AU	0.3

AMC- Amoxicillin-clavulanic acid, TE- Tetracycline, AMP- Ampicillin, CPX- Ciprofloxacin, CFT- Ceftriaxone, SP- Sparfloxacin, AU- Augmentin and APX- Ampiclox

Table 4 Antibacterial activity of silver nanoparticles synthesized by *B. thuringiensis* isolate CR2³ against some selected drug resistant bacteria

Test Isolates	AgNPs				Controls	
	Mean zone of inhibition (mm) at different concentrations (μg/mL)					
	100	75	50	25	CP*	Bt culture**
<i>E. coli</i> (strain 1)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
<i>E. coli</i> (strain 2)	19.0±1.6	13.0±0.4	13.0±1.6	13.0±1.5	30.0±0.8	0.0±0.0
<i>K. pneumoniae</i>	15.0±0.4	14.0±0.8	14.0±0.8	13.0±0.4	20.0±0.4	0.0±0.0
<i>S. aureus</i>	17.0±0.8	15.5±0.4	14.0±0.4	13.5±0.4	40.0±0.8	0.0±0.0

*CP-Ciprofloxacin (10 μg/mL); ** Negative control- AgNO₃ -free Bt culture

The potential biomolecules responsible for the reduction of Ag⁺ into Ag⁰ NPs were identified by FTIR spectroscopy (Liaqat et al. 2022). FT-IR measurement was carried out to identify the possible interactions between silver and bioactive molecules, which may be responsible for synthesis and stabilization (capping) of AgNPs. The broad peaks at 3379 and 1643 cm⁻¹ correspond to the existence of amine and amide I group respectively indicating that proteins were the capping and stabilization biomolecules in the synthesis of AgNPs (Shankar et al. 2014). The broadness of the peak

Table 5 Antibacterial activity of silver nanoparticles synthesized by *B. thuringiensis* isolate MRS2¹ against selected drug resistant bacteria

Test Isolates	AgNPs				Controls	
	Mean zone of inhibition (mm) at different concentrations (µg/mL)				CP*	Bt culture**
	100	75	50	25		
<i>E. coli</i> (strain 1)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
<i>E. coli</i> (strain 2)	19.0±0.8	15.0±0.7	13.0±1.5	12.5±0.8	30.0±0.4	0.0±0.0
<i>K. pneumoniae</i>	22.0±0.2	20.0±0.2	19.0±0.8	19.0±0.4	20.0±0.4	0.0±0.0
<i>S. aureus</i>	20.0±0.3	16.0±0.3	15.0±0.0	11.0±0.2	40.0±0.0	0.0±0.0

*CP-Ciprofloxacin (10 µg/mL); ** Negative control- AgNO₃ -free Bt culture

Table 6 MIC and MBC of AgNPs against the selected drug resistant bacteria

Test organisms	AgNP (MRS2 ¹) (µg/mL)		AgNP (CR2 ³) (µg/mL)	
	MIC	MBC	MIC	MBC
<i>E. coli</i> (test strain 2)	50±0.0	75±0.0	50±0.0	100±0.0
<i>S. aureus</i>	50±0.0	100±0.0	50±0.0	100±0.0
<i>K. pneumoniae</i>	25±0.0	100±0.0	50±0.0	100±0.0

could be as a result of the overlap of both O-H and N-H bond stretching of primary and secondary amines (Ojo et al. 2016). The peaks 1265, 2137, and 2407 cm⁻¹ are assigned to the O-H vibration of alcohols, C≡C stretch of alkynes, and C-N of nitrogen compounds respectively. These indicate that biomolecules rich in amine (N-H) and hydroxyl (O-H) groups were responsible for the reduction of Ag⁺ to Ag⁰, as well as capping of AgNPs to prevent their agglomeration (Elamawi et al. 2018). It is therefore evident that proteins present in the cell-free extract of Bt isolate CR2³ culture supernatant accounted for the capping and stabilization of the AgNPs. It is important to understand though, that it is not just the size and shape of proteins, but the conformation of protein molecules that plays an important role in stabilization of the AgNPs produced (Jain et al. 2010).

The proposed mechanism of action of AgNPs on bacteria includes its ability to interact with membrane phospholipids, cell membrane rupturing, and physical interference with cellular components, ROS generation, interaction with cytosolic proteins and enzymes, and elevated metal ion concentration. The potential of AgNPs to act as an antibacterial is due to membrane disruption of microbes with adhesive substances like proteins, polysaccharides and the bactericidal action of Ag⁺ ion (Liaqat et al. 2022).

Previous studies demonstrated that silver nanoparticles can act by inactivating both Gram- positive and negative drug resistant and drug susceptible bacteria, exerting bactericidal activity and inhibiting bacterial growth

following contact with nanoparticles (Lara et al. 2010). AgNPs may interact with bacterial cell membrane and form aggregates and causing cell damage (Sondi and Salopek-Sondi 2007). Antimicrobial activity of NPs may be affected by shapes of NPs which interact with periplasmic enzymes causing varying gradations of bacterial cell damage (Cha et al. 2015; Varier et al. 2019). Although there are currently no reports on the antibacterial activity of anisotropic shaped NPs seen in this study, there are reports and activities of other shapes. Actis et al. (2015) reported that cube-shaped AgNPs exhibit stronger antibacterial activity than sphere-shaped and wire-shaped AgNPs with similar diameters, due to the specific surface area and facet reactivity. However, Yao et al. (2013) compared the antibacterial activity of polymer nano-objects with sheet-like, cylindrical, and spherical shapes and found no significant difference in antibacterial performance across the series. Therefore, apart from size, other factors such as virulence of the tested organism or shape of the NPs could be responsible for the efficacy AgNPs as antimicrobial agents. The extensive use of AgNPs in a range of consumer products such as cosmetics, childcare products, food packaging and appliances raise concerns about the development of silver-resistant bacteria. Also, following exposure to toxic heavy metals such as silver, co-selection may induce the emergence of antibiotic resistance. AgNPs have multiple targets of mechanism hence antibiotic resistance mechanisms are not expected to influence their activity (McNeilly et al. 2021).

To the best of our knowledge, this is the first report on the extracellular synthesis and antibacterial activity of AgNPs from *Bacillus thuringiensis* isolated from soils in Nigeria. The limitations include difficulty in obtaining standard AgNPs, hence it was not used as a control. We did not conduct NPs size measurement using other techniques like X-ray Diffraction and 16 S RNA sequencing of the *B. thuringiensis* was not conducted due to limited funds. Hence, further analyses are recommended in future studies to provide more information on the properties of the NP and *Bacillus thuringiensis*.

Conclusion

AgNPs synthesized extracellularly from *Bacillus thuringiensis* isolated from soils exhibit antibacterial activity against some drug resistant pathogens. There is need to assess the effect of irregular and anisotropic AgNPs on their antibacterial activity. Further studies on the synergistic effect of these AgNPs and antibiotics are required for developing new strategies for combating antimicrobial resistance and reducing their global public health burden.

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Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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