

RESEARCH PAPER

## Biosynthesis of Platinum Nanoparticles by *Streptomyces fradiae* and Evaluate Antibacterial Activity

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### ABSTRACT

The present study aimed to isolate the bacterial strain *Streptomyces fradiae* from soil and screen it for its ability to biosynthesize PtNPs and characterize and evaluate the antibacterial potential of the manufactured PtNPs. *Streptomyces fradiae* was isolated from soil samples using the serial dilution method. The isolated bacteria were identified using a light microscope, then diagnosed by biochemical examinations, and lastly by the Polymerase chain reaction (PCR) for molecular diagnostics. The results confirmed the presence of *Streptomyces fradiae* depending on the amplification of 16s RNA fragments. That was followed by the bacterial screening to examine their ability for use in the production of platinum nanoparticles. The formation of biogenic platinum nanoparticles was confirmed by physical characterization for example UV-VIS spectrophotometer exposed the presence of UV spectrophotometer peak in wavelengths at 362 nm. The results of (TEM) in the electron transmission microscope exposed that the biogenic platinum NPs were in sizes between (2.44-29.57) nm. The field emission scanning microscope also exhibited the examination of surface morphology, shape, and size, where the average diameter for PtNPs was calculated and the final value for this diameter was 5.55 nm. The Zeta potential analyses showed that platinum nanoparticles gained negative surface charges at -28.6 mV. The antibacterial activity of the platinum nanoparticles was evaluated against pathogenic bacteria, there were five species isolated from the hospital, and they exhibited an inhibitory effect on the tested bacterial isolates.

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### INTRODUCTION

*Streptomyces* is a genus belonging to the Actinomycetes group, which represents a Gram-positive filamentous bacteria existing in marine and terrestrial habitats and one of the largest taxa in bacteria, they are notable for their complicated and fungal-like life cycles [1]. They have a supreme ability to produce various active compounds like antibiotics, the studies indicated

that this genus provides approximately 80% of the industrially antibiotics [2]. Recently, inorganic nanoparticles (NPs) have gained vast research interest in the science and technology fields [3-5]. Nanotechnology generates a diversity of nanoscale materials with a size of about (1–100) nm, these nanoscale materials are known as nanoparticles NPs [6]. Nanotechnology is a current practice that involves the production, characterization,

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and application of nanoparticle NPs [7]. It has progressed significantly in recent decades because of the fabrication and various applications of NPs in numerous fields like biology, engineering, agriculture, electronics, cosmetics, and medicine [8,9].

Nanoparticles (NPs) have gained much attention because of their distinctive physicochemical properties and significant medical applications [10]. Numerous methods have been used to fabricate the nanoparticles (NPs) including physical, chemical, and biological ways [11-14]. Modern studies have concentrated on the biosynthesis of NPs and especially bacterial biosynthesis due to some properties, involving the simplicity of cultivating bacteria, easy experimental settings for instance, (pH, and temperature) and short generation time [15]. Also, studies have shown that bacterial cells play an essential role in the transformation of heavy metals to nanoparticles. An additional advantage is their ability to synthesize enormous quantities of long-lasting nanoparticles [16].

The metal nanoparticles involving gold, silver, titanium, copper, platinum, and palladium have been showed to be applied in a lot of different applications such as chemistry, biology, ecology, and medicine [17-25]. Among these nanoparticles platinum nanoparticles (Pt NPs) have generated special interest due to their unique structural, catalytic, and optical properties, also because of their high surface area, and good corrosion resistance which makes them a potential candidate for catalysis and biomedical applications [26,27]. Platinum nanoparticles have been showing major success in numerous applications where it is reckoned to be competent and can serve as drug carriers [28]. Moreover, platinum nanoparticles Pt NPs on a variety of medical uses, including anti-cancer, anti-oxidant, anti-diabetic, antibacterial, and antifungal applications [29]. Actinomycetes represent a Gram-positive filamentous bacteria existing in marine and terrestrial habitats. They have a supreme ability to produce various active compounds like antibiotics. Studies have shown that Over 500 species of actinomycetes have been described, till now, and among them, *Streptomyces* genus provides approximately 80% of the industrial antibiotics [2].

The biosynthesis of nanoparticles (NPs) by Actinomycetes has been reported and it has many advantages such as good stability and

polydispersity, also they can be genetically manipulated to provide better control over the size of the nanoparticles. Actinomycetes are known to convert the metal salts to metal nanoparticles NPs extracellularly and intracellularly. however, the synthesis mechanism of NPs differs depending on the three a countless potential application of the green synthesized PtNPs such as anti-microbial, anti-parasitic, antioxidant, and anti-cancer therapies have been testified. NPs are active against various microbial species and exhibit minimal bacterial resistance which makes them effective as anti-bacterial agents, many studies have shown an effective use of metal ions that can damage bacterial DNA, cell membranes, and essential enzymes and kill bacteria during a process termed as respiratory burst mechanism [30]. The present study aimed to isolate the bacterial strain *Streptomyces fradiae* from soil and screen it for its ability to biosynthesize PtNPs and characterize and evaluate the antibacterial potential of the manufactured PtNPs.

## MATERIALS AND METHODS

### Soil Samples Collecting

Thirty Soil samples were gathered from several locations, including sugar cane fields and gardens. Samples were taken from a depth of (11 mm). The soil was dug with a trowel, and the samples were gathered in sterile tiny plastic bags that were clearly labeled with the date and place of collection. Then the soil samples were dried for three hours in a hot air oven at 60°C, the dried samples were transferred to tubes and reacted with 0.2 g of CaCO<sub>3</sub> and keep at 4°C. After that the samples will diluted by following the procedure of dilution method step by step and cultures on a starch-casein-nitrate agar media (SCN) as an isolation medium, the pH was checked before being sterilized in 121°C autoclave for 15 minutes, and purified in the selective medium (Yeast Extract Glucose agar); the procedure according to [31].

### Identification of the isolates

Several methods were used in the identification of the isolates [31], firstly through the morphological methods of identification, and the biochemical tests methods, and secondly by the molecular identification, in which the DNA of the isolates extracted using Presto™ Mini gDNA bacteria kit according to the manufacturer instructions. The genomic DNA was stored at

-20°C. All bacteria isolated by gene 16SrDNA (1500 bp) gene by amplified by thermocycling device General prefixes Front starter (5'-GATGACGTCAAATCATCATGC-3') and the reverse initiator (3'-AGGAGGTGATCCAGCCGCA-5'). The reaction mixture was carried out with a volume of 20 µL, where a DNA mold, primers, and distilled water were added to each tube of GoTaq<sup>®</sup>G2 Green Master Mix is a ready-to-use mixture of high-quality Taq DNA Polymerase, deoxynucleotides, and reaction buffer in a 2X concentration. It contains all the necessary reagents for the amplification of DNA. The GoTaq<sup>®</sup>G2 Green Master Mix contains an inert green dye and a stabilizer which permit direct loading of the final products onto a gel for analysis. Each sample was sent to the South Korean biotechnology company Macrogen for purification as a product and sequencing analysis for the general initiator Next, the sequence results were compared with the sequences of the front- and reverse prefixes by the same company. (<https://www.ncbi.nlm.nih.gov>) It is then analyzed to detect the closest match to bacterial isolates.

#### *Biosynthesis of PtNPs from studied bacteria*

Platinum nanoparticles were prepared following the method described by [32]. Bacterial strains that have been isolated, purified, labeled, and cultured in MGYB broth and nutrient broth (pH 7) at 29°C for 7 days with constant shaking on a shaker incubator (150 rpm). After the incubator time, it was filtered through Whitman filter paper (No.1) and also in the centrifuge (14000 rpm) to obtain the supernatant. The solutions used in the synthesis of PtNPs, the first is made by mixing (v/v) 100 ml of supernatant with 100 ml of chloroplatinic acid hydrate (2mM). As a control, a second reaction mixture is made without chloroplatinic acid hydrate. The second solution was made by mixing 50ml of supernatant with 10ml of PtCl<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>, in both ways the solutions were kept on a rotary shaker (150 rpm) at 29°C for 1-2 weeks in the dark (to prevent photochemical reversal during the experiment). The first indicator for the synthesis of PtNPs is the appearance of a color change in the culture solution as a sign of the development of Bio-PtNPs [33].

#### *The physical Characterization of PtNPs*

##### *UV- Spectroscopy*

A UV-visible spectral scan at 200-800nm was used to ensure the creation of platinum NPs. The

solutions were centrifuged for 5min at 2000 rpm. The untreated supernatant was set as reference control (blank) while treated supernatants were used to screen their UV-visible absorbance Spectra between 200-800 nm wavelength [34].

##### *Fourier transform infrared spectroscopy (FTIR)*

The FTIR analysis was done to identify the presence of functional groups in the fabricated platinum NPs, the functional groups are very important and facilitate the biosynthesis process. By the FTIR spectrometer the samples (in powdered form) were measured in the range of 400–4000 cm<sup>-1</sup> [35].

##### *XRD Analysis*

X-ray diffraction (XRD) is one of the most widely used techniques for characterizing PtNPs. XRD usually provides information concerning crystal structure, lattice dimensions, phase nature, and crystal sizes [23]. The samples were measured by X'pert Pro X-ray diffract meter, the diffraction pattern of the powdered form of biogenic NPs was recorded from 10° to 80° (2 theta), with a step size of 0.050°, by Cu K-Alpha radiation (k = 1.54060 Å) and working at 40 kV and 30 mA. Scherer's equation was applied to find the average crystalline size of the NPs as previously described.

##### *Transmission electron microscopy (TEM)*

TEM was used to analyze the size, shape, and distribution of synthesized nanoparticles (PtNPs) [36].

##### *Atomic force microscopy (AFM)*

The AFM device was used to characterize the size and morphology of the platinum NPs. Thin films of bio-fabricated NPs were coated on clean glass coverslips before the AFM scanning and permitted to dry at room temperature [37].

##### *Zeta potential analysis*

The zeta potential method was used to measure the stability of the platinum NPs using a zeta potential analyzer instrument, the samples were centrifuged, and the NPs were measured between -200 and +200 mV at 25.2°C (temperature of the holder) [38].

##### *Field Emission Scanning Electron Microscope (FESEM)*

SEM is a surface imaging technique in which

an incident electron beam scans over the specimen surface and interacts with it, generating signals that represent the specimen's atomic composition and topographic information. SEM generates many better-resolution images by using accelerated electron beams and electrostatic or electromagnetic lenses [39].

#### *Antibacterial activity of bio-synthesized PtNPs*

The antibacterial activity of the manufactured PtNPs was examined using the method (agar disc diffusion) against several pathogenic bacteria isolated from clinical specimens. The specimens were isolated from patients in AL-Sadr Hospital in Maysan Governorate, Iraq. Five species of pathogenic bacteria, including four Gram-negative bacteria (*Proteus mirabilis*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *E. coli*), and one Gram-positive bacteria (*Staphylococcus aureus*) were tested. In nutrient agar, pure colonies of bacteria were grown at 37°C for 24 hrs., and the turbidity was adjusted to 0.5 McFarland standard using sterile distilled water. Each type of bacteria was uniformly swabbed onto MHA plates, then we put PtNPs discs. After that the discs were gently press down to guarantee contact. The plates were incubated right away. Petri dishes were evaluated for the inhibitory zone measure in millimeters after incubation for 24 hours at 37°C. As a control

gentamicin disc (10 µg) was used to compare with PtNPs discs [40,41].

## RESULTS AND DISCUSSION

### *Isolation of studied bacteria*

During this study, the results were collected from soil sources. The isolated bacteria were cultured on a starch-casein-nitrate agar media (SCN) as an isolation medium, and purified by transplantation on the selective medium (Yeast Extract Glucose agar). This medium is suitable for the growth and extraction of these bacteria. The colonies were characterized depending on their differences in colony, morphology, and other features like variations in colors (black, white, pink, gray, purple, and yellow), and the variety in their texture from powdery to gelatinous. The morphological characterization was used to distinguish between Actinomycetes and other bacteria, as shown in Fig. 1.

### *Molecular identification of the isolated bacteria*

The determination of the identities of bacterial isolates was done by polymerase chain reaction (PCR) technique through the amplification of the 16S rDNA gene (1500 bp). the 16S rDNA gene sequence exposed that the isolated bacteria was comparable to Gen Bank. The BLAST program was used to analyze the DNA sequencing results of

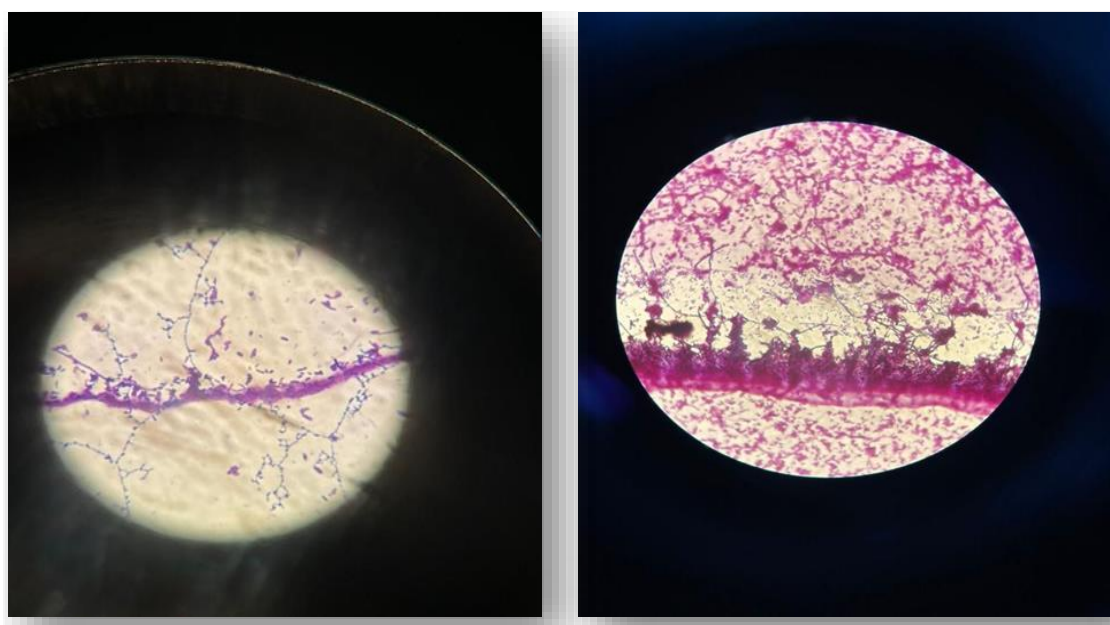


Fig. 1. Actinomycetes isolates staining by gram stain

the bacterial isolates and match them with their reference strains in the Gen Bank. The Gen Bank database preserves and stores the nucleotide sequences of the genomic DNA [42]. In the current study, the NCBI BLASTn search engine showed a high 99% sequence similarity between the sequenced samples and the reference target sequences of *Streptomyces fradiae*. By comparing the specific DNA sequences of these examined samples with those of *Streptomyces fradiae* (GenBank acc. MN901087.1) the accession no. of closet bacteria. Other details were determined, the exact locations of the recovered PCR fragments, and the total length of the target site were analyzed using the NCBI server, and the locations of the target site within the most matching bacterial target gene were confirmed.

*Bacterial screening for the ability to synthesize PtNPs*

After growing the isolated bacteria from soil samples on the fermentation media (MGYP and nutrient broth) and by obtaining bacterial filtrate (free cell extract), which was mixed with chloroplatinic acid hydrate dissolved in deionized water, the results showed the presence of color change after an incubation period in *Streptomyces fradiae*. This confirms a reaction between the active secondary compounds in the bacterial extract with the chloroplatinic acid hydrate. In addition, this means that these two species own the ability to biosorb metal ions on their

surface and reduce them to the corresponding nanoparticles through different mechanisms by enzymatic activity which may include reductases, cytochromes, and metallothioneins [43].

The reaction mixture showed dark/greyish brown color upon reaction, Fig. 2 indicating the formation of platinum nanoparticles. Chloroplatinic acid hydrate was added to the cell free supernatant (CFS) and the color changed from pale yellow to greyish brown that was observed after (7-10) days of reaction. The color changes that occurred are due to the phenomenon of Plasmon Surface Resonance. These results agreed with the study of [32].

*Characterization of the synthesized platinum nanoparticles*

*UV-Vis. Spectroscopy*

UV-Vis spectroscopy was used immediately after the reduction of platinum, and the ultraviolet-visible spectrometric measurement were performed in the range of (200-800) nm to confirmed the formation and stability of platinum nanoparticles [44]. The reduction was from Pt<sup>4+</sup> to Pt<sup>0</sup>, and the color of the solution was observed to change from yellow to greenish brown, which indicates that distinct platinum nanoparticles were formed as a result of the reduction of H<sub>2</sub>PtCl<sub>6</sub>, Fig. 3 represented platinum nanoparticles synthesize laboratory using *Streptomyces fradiae*, and the broad peak of this nanoparticles (362) nm wavelength [28,48].

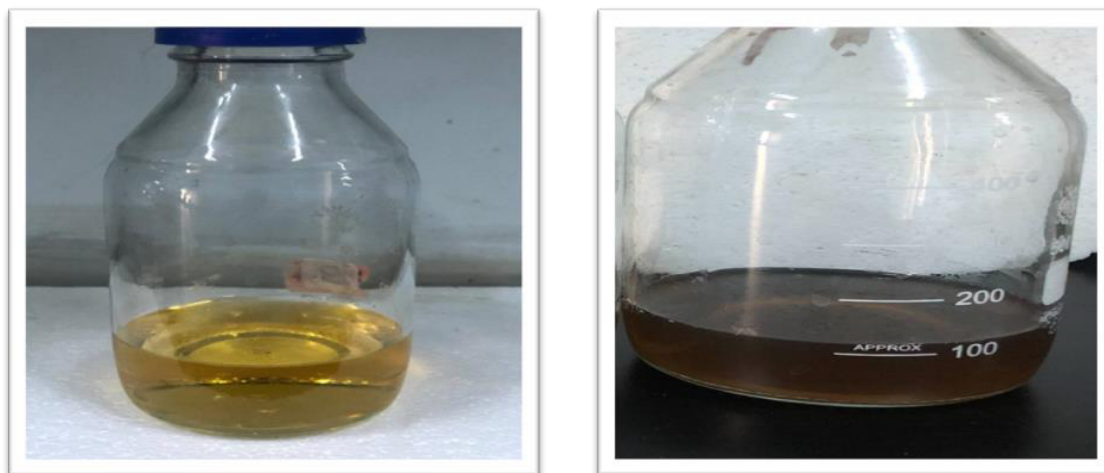


Fig. 2. The color change of the culture supernatant of the bacterial strain *Streptomyces fradiae*.

The Fourier Transform Infrared Spectroscopy (FTIR) FTIR spectra were working to discover the different functional groups of the biosynthesized PtNPs that were involved in the reduction of

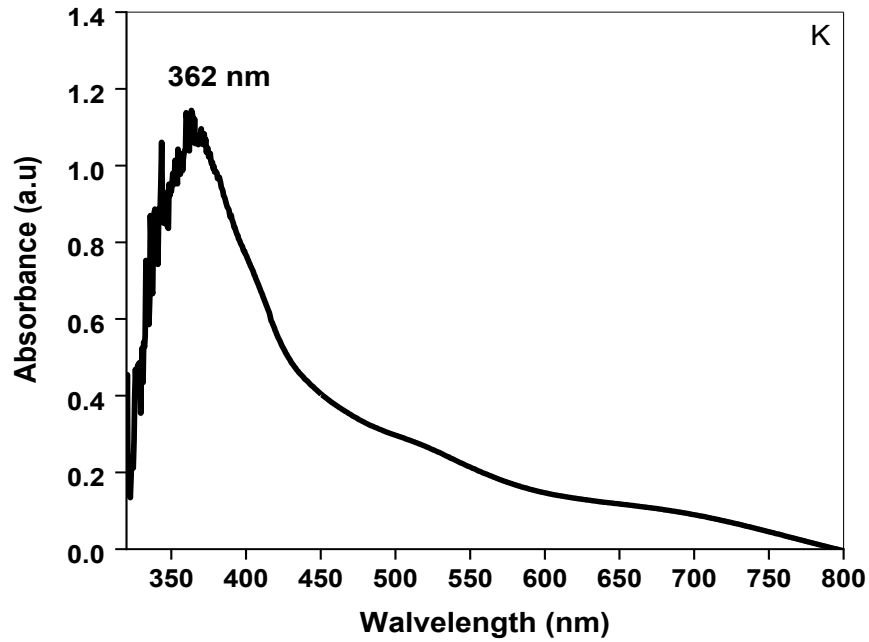


Fig. 3. UV-visible spectroscopy of platinum nanoparticles synthesized by *Streptomyces fradiae* which showed the sharpest peak of UV-Vis spectrum at  $\lambda_{max} = 362$  nm.

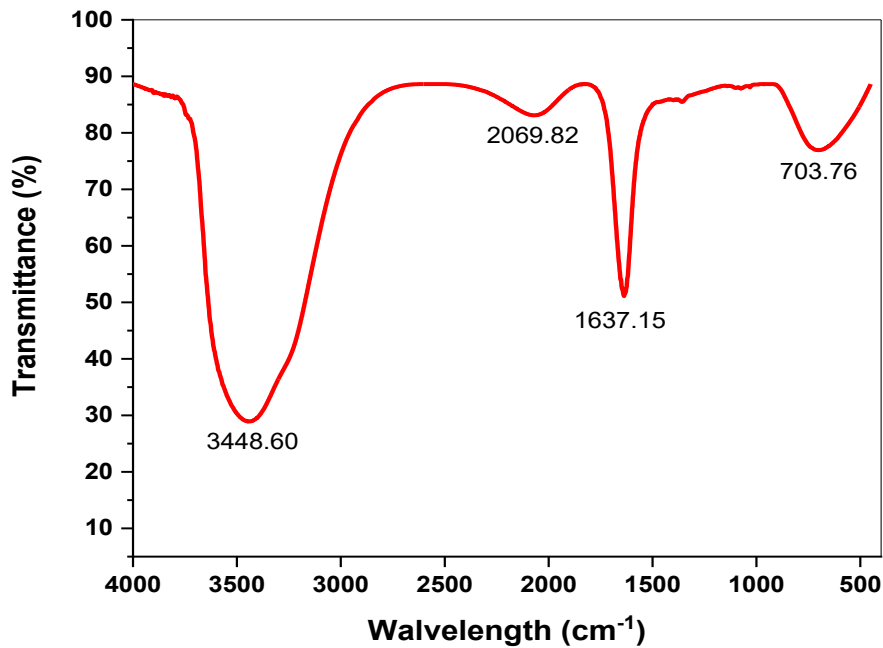


Fig. 4. FTIR spectroscopy spectrum of biosynthesized PTNPs using *Streptomyces fradiae*



precursors to NPs, it can be concluded from Fig. 4 that the functional groups help in the reduction process and stabilization of the biosynthesized platinum nanoparticles. In the present study, the FTIR spectrum of platinum nanoparticles

synthesized by *Streptomyces fradiae*, the FTIR spectrum of these nanoparticles shows the appearance of different peaks at  $3448\text{ cm}^{-1}$ ,  $2069\text{ cm}^{-1}$ ,  $1637\text{ cm}^{-1}$ , and  $703\text{ cm}^{-1}$ . The peak at  $3448$  and  $1637\text{ cm}^{-1}$  may be attributed to the  $(-C=C-$

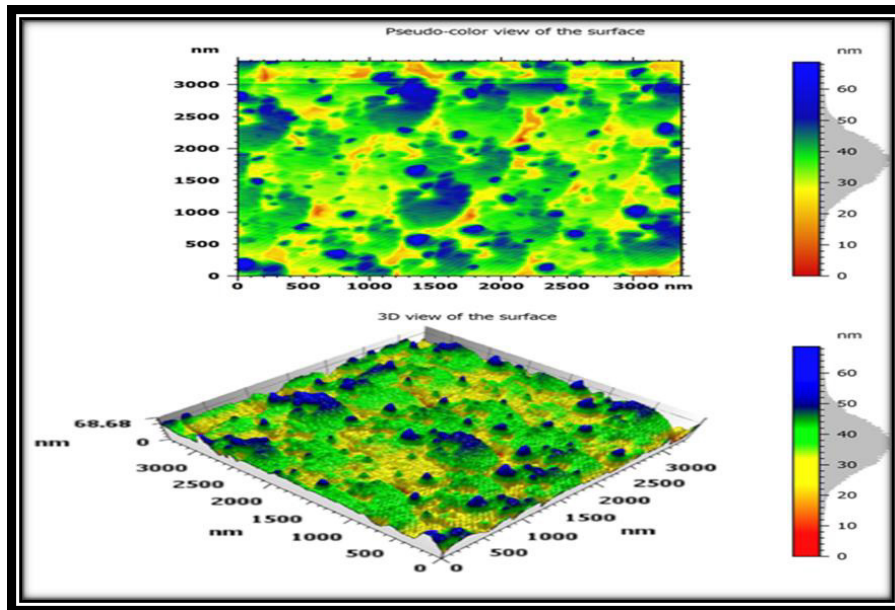


Fig. 5. 2D and 3D images of AFM for PtNPs synthesized by *Streptomyces fradiae*

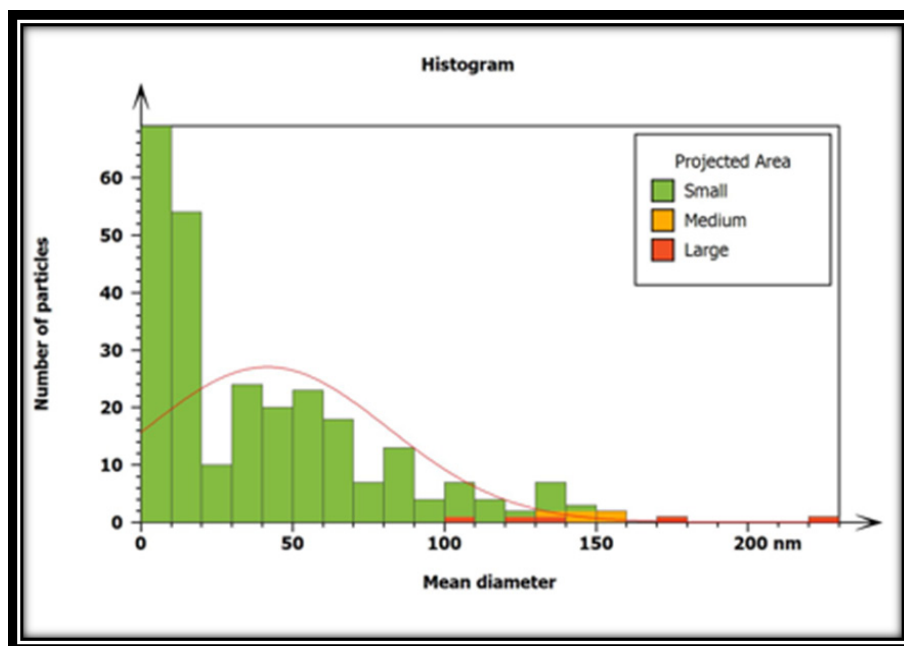


Fig. 6. The mean diameter of AFM for PtNPs produced by *Streptomyces fradiae*

and free N-H) vibrations, which could be assigned to the heterocyclic compounds like proteins. The peak of  $2069\text{ cm}^{-1}$  refers to the presence of the amines functional group ( $\text{R-N=C=S}$ ). while the peak of  $703\text{ cm}^{-1}$  is assigned to alkyl halides ( $\text{C-X}$ ), the functional group chloride [45].

#### Atomic force microscopy (AFM)

Atomic force microscopy is used to study and understand the shapes, topography, roughness, and protrusions of surfaces through this examination, in which the particles are represented by their heights and surface structure, which makes it

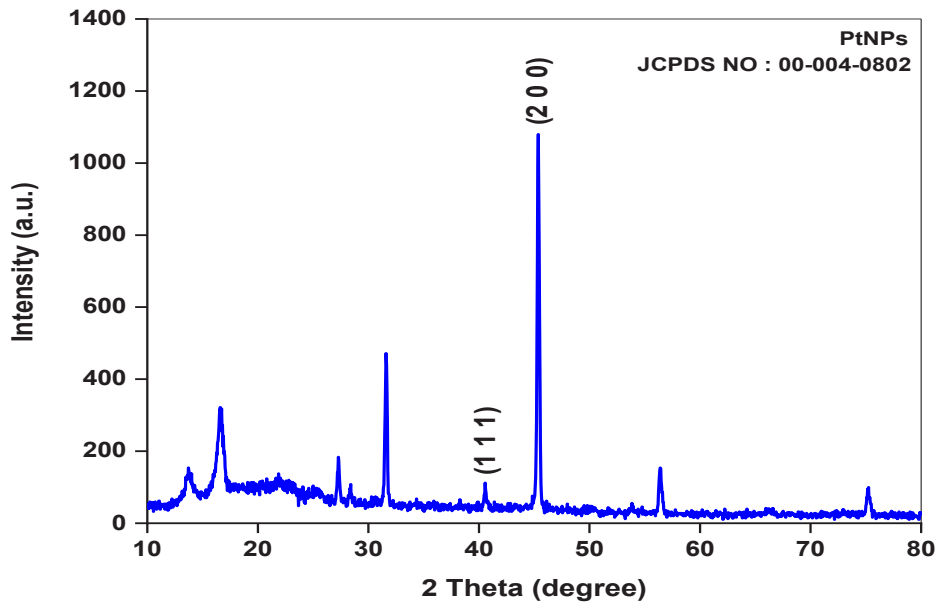


Fig. 7. XRD analysis of PtNPs produced by *Streptomyces Fradiae*

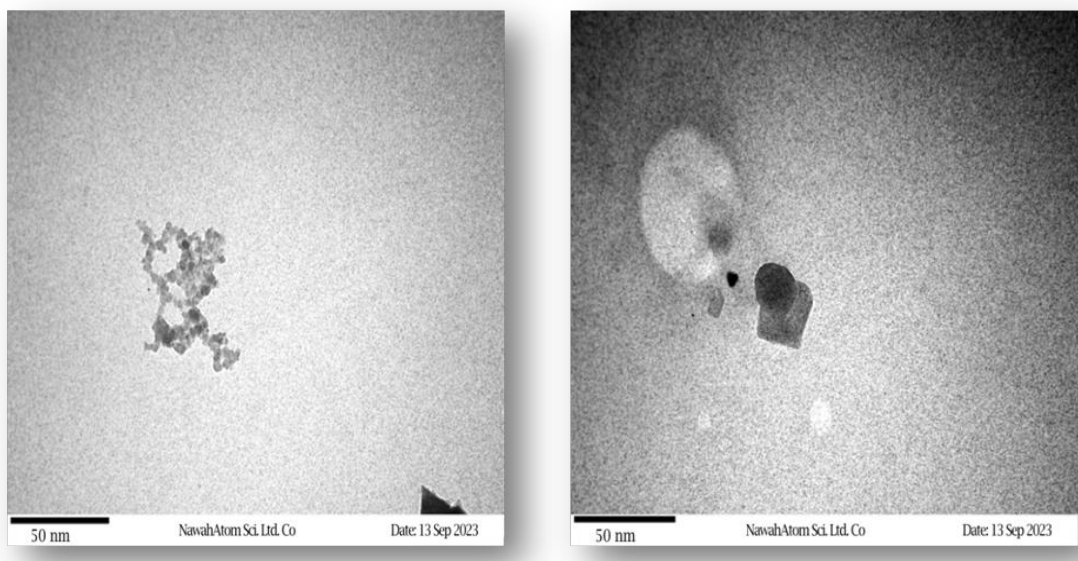


Fig. 8. The TEM images of PtNPs synthesized using *Streptomyces Fradiae*



possible to perform quantitative measurements to know the surface structure and access two- and three-dimensional images and analyze them from different sides. The AFM results for PtNPs

produced using *Streptomyces fradiae* were that PtNPs had a mean diameter (of 41.66 nm), and the particle size was between (4.89- 227.1 nm), the two-dimensional (2D) and three-dimensional

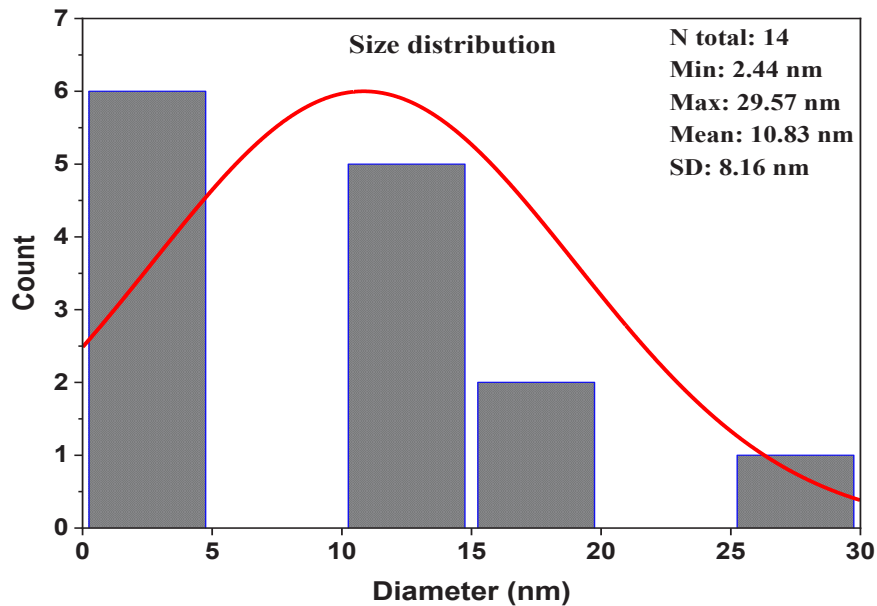


Fig. 9. The size distribution of TEM

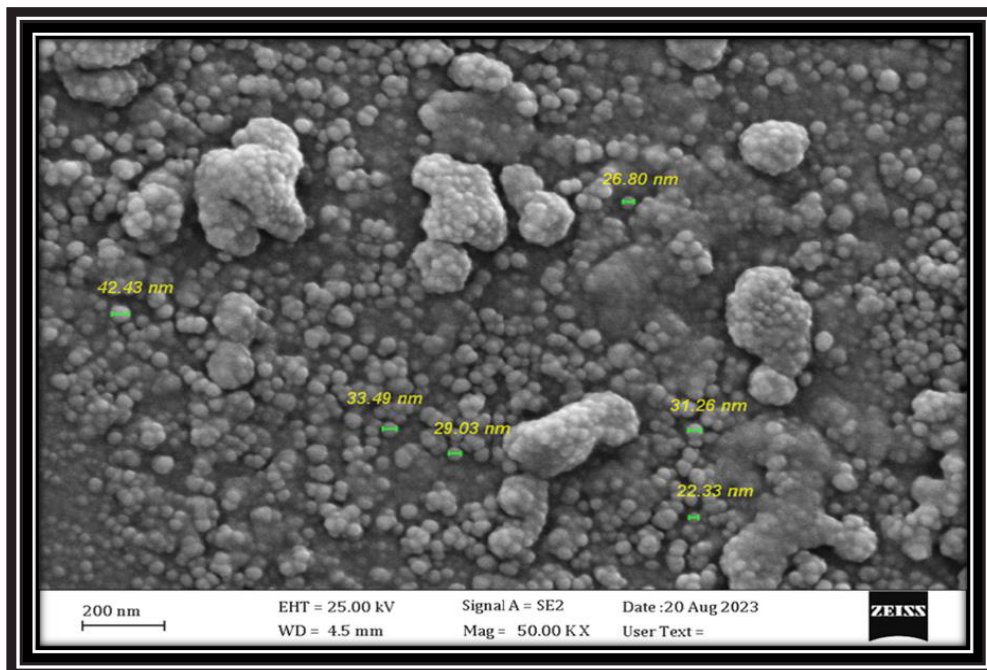


Fig. 10. The FESEM image of PtNPs synthesized using *Streptomyces fradiae*

(3D) images of AFM shown the spherical shape and the uniform distribution of the synthesized PtNPs achieved by TEM and FESEM micrographs as presented in Figs. 5 and 6. We could consider the surface of the material as a rough surface

based on atomic force microscopy (AFM) images because it contains peaks of different dimensions. The presence of surface roughness will increase the effectiveness of killing bacteria and cancer cells [25].

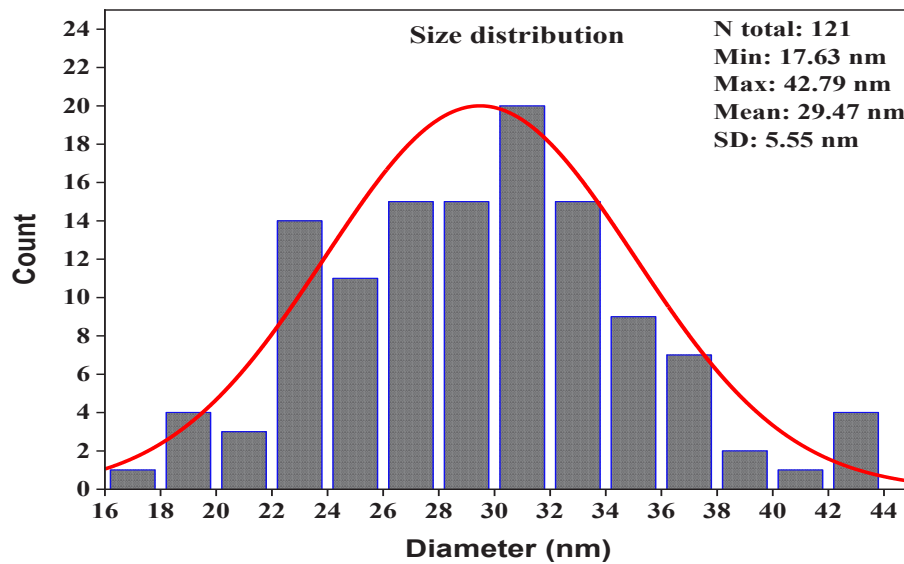


Fig. 11. The histogram of the size distribution of *St. fradiae*.

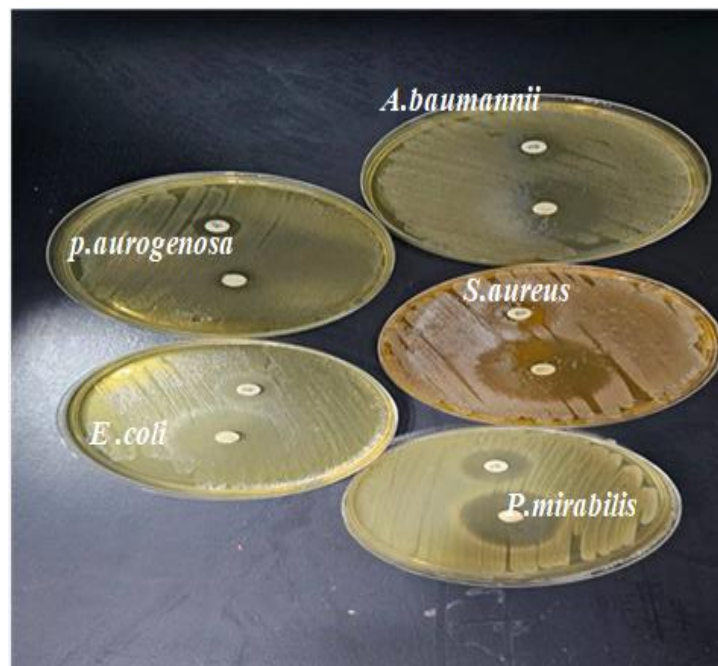


Fig. 12. Inhibition Zone on five Pathogenic Bacteria by PtNPs and Gentamycin Antibiotic as a control.

### XRD Analysis

The analysis of the structure and crystalline size of the platinum nanoparticles (PtNPs) was carried out by XRD (Fig. 7). The XRD analysis of PtNPs biosynthesized by *Streptomyces Fradiae* shows that the peaks were observed at  $2\theta = 40.51, 45.39$  corresponding to the lattice planes of the crystalline structure of PtNPs (111), and (200), respectively; these outcomes are in agreement with the Joint Committee on Powder Diffraction Standards (JCPDS) file NO. 00-004-0802. The XRD showed that the particles synthesized using the green method have a cubic crystal structure.

### Transmission Electron Microscopy (TEM)

The size and morphology of the PtNPs were observed by the TEM analysis. The TEM image of platinum nanoparticles biosynthesized using *Streptomyces fradiae* (K) confirmed that the nanoparticles were spherical, non-agglomerated, monodisperse and the sizes were between (2.44-29.57) nm, with mean particle size 10.83 nm and size distribution of about 8.16 nm. It was evident that the biologically synthesized PtNPs by *Streptomyces sp.* have somewhat uniform spherical shapes with well distributed within the size range of 20–50 nm as shown in Figs. 8 and 9. The size ranges obtained from the TEM results also agreed with the other topographical and analytical results [46].

### Field emission Scanning Electron Microscope (FESEM)

The surface morphology, composition, size, and shape of platinum nanoparticles synthesized

from the isolated bacteria under study were examined by field emission scanning electron microscopy (FESEM). The FESEM results of PtNPs biosynthesized using *Streptomyces fradiae* confirm that the particles were spherical with the size between (17-42) nm, the size distribution was 5.55 nm. This agreed with many studies that showed the size of synthesized platinum nanoparticles was between (20–30) nm, and the NPs were agglomerated. Which could be to perform a more evaluation of the shape, size, and morphologies of the nanoparticles (Figs. 10 and 11).

### Antibacterial activity of biosynthesized PtNPs

The antimicrobial activity of biosynthesized PtNPs was studied against five species of pathogenic bacteria isolated from the hospital, which were: *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Acinetobacter baumannii* (Fig. 12). The antimicrobial activity compared with Gentamicin antibiotic is standard for these pathogenic bacteria [41]. Four strains were inhibited by PtNPs except *Pseudomonas aeruginosa*. The sizes of bacterial inhibition zones vary from pathogenic bacteria to another as shown in Table 1. The primary issue for public health is that bacterial strains identified from various ecosystems are resistant to antibiotics used in human medicine, severely limiting therapeutic options and endangering affected people's lives [47].

### CONCLUSION

The primary aim of this investigation was to develop a novel method for producing platinum

Table 1. Antibacterial Activity of PtNPs Biosynthesized by *Streptomyces Fradiae* Against Pathogenic Bacteria Isolated from the Hospital

NO	Pathogenic bacteria	Inhibition zone (mm) of PtNPs	Inhibition zone (mm) of Gentamicin antibiotic
1	<i>Escherichia coli</i>	10.3	4.1
2	<i>Proteus mirabilis</i>	15.2	7.2
4	<i>Staphylococcus aureus</i>	16.4	7.1
5	<i>Acinetobacter baumannii</i>	10.2	4.3

nanoparticles utilizing the *Streptomyces fradiae* strain of bacteria. To begin, the selected *Streptomyces fradiae* bacteria submit to identification procedures, including a series of chemical tests complemented by the extraction of DNA for molecular identification. Following successful identification, the research progressed to the synthesis phase, where platinum nanoparticles were produced by the bacteria. The resulting platinum nanoparticles (PtNPs) were then subjected to a wide structural examination employing a range of advanced analytical techniques, such as UV-visible spectroscopy, field emission scanning electron microscopy (FE-SEM), atomic force microscopy (AFM), transmission electron microscopy (TEM), and X-ray diffraction (XRD). In addition to practice, in different applications, the antibacterial activity of these nanoparticles was evaluated through their interaction with various pathogenic bacterial species to evaluate any potential inhibitory effects. In this study, the biosynthesized PtNPs demonstrated significant antibacterial activity against the tested pathogens. Given these findings, it is very important for future research to focus on exploring a broader spectrum of biological activities that PtNPs may influence, or to consider new applications for these nanoparticles or shed light on the nuances of their interaction with biological entities.

#### CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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