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Biosynthesis of Platinum Nanoparticles from Some Actinomycetes and Study Their Biological Activity

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By

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

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Acknowledgment

Praise God as he deserves it, and praise God who had mercy on us with Muhammad, his Prophet. May God's prayers and peace be upon him and his family. And after:

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Zainab

Dedication

To my beloved country

To the martyrs of Iraq

To my dear father and beloved mother

To my companion, my husband, and my beloved children 'Jaafar, Rafal, Mayar.'

To my dear uncles, brothers, and sisters

I dedicate my simple work...

Abstract

The present study focuses on the biosynthesis of platinum nanoparticles using an easy, friendly, low-cost, environmentally friendly method using the supernatant of the isolated bacteria from the soil. Nanobiotechnology is a current field that agrees with the progress of technology at the molecular or atomic level, in which structures with a length scale in the range of (1 - 100) nanometers have been processed and studied.

This study obtained thirty soil samples from locations in Misan Governorate from October 2022 to December 2022, including rice fields, home gardens, and agricultural lands. The soil samples were brought to the Biotechnology Laboratory at the College of Science at Misan University. The bacteria were isolated using the serial dilution method. Cultured on Starch-Casein-Nitrite Agar media and purified on Yeast Extract Glucose Agar (selective media). 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. After that, the bacterial isolates were screened to examine their ability to produce platinum nanoparticles .The procedure was to mix the bacterial filtrate with a chloroplatinic acid hydrate (H2pt2cl6) solution. The formation of biogenic platinum nanoparticles was confirmed by physical characterization. The biosynthesized platinum nanoparticles were screened for their toxicity against pathogenic bacteria, cancer cell lines, and normal cells.

The results showed there were six isolates of Actinomycetes from bacteria they were: *Streptomyces omiyaensis*, *Streptomyces macrosporeus*, *Streptomyces thermolilacinus*, *Streptomyces fradiae*, *Streptomyces zaomyceticus*, *Streptomyces ziwulingensis* isolates characterized depending on their differences in the colony, morphology and other features. Concerning the ribosomal amplified fragments, the NCBI BLASTn engine showed an entire sequence similarity of 100% between the five samples (assigned Z1, Z2, Z3, Z4, and Z12) and the nucleic acid sequences of *Streptomyces* sp. In contrast, one sample (assigned Z8) showed 99% similarity with

the same target. The supernatant was mixed with platinum salt to produce platinum nanoparticles, and the results showed a colour change for two bacterial isolates as an indicator of the production of platinum nanoparticles. The results of the UV-visible spectrophotometer that exposed the presence of UV spectrophotometer for Platinum nanoparticles biosynthesized using *Streptomyces fradiae* showed a broad peak of these nanoparticles (362) nm wavelength, while platinum nanoparticles biosynthesized by *Streptomyces ziwulingensis* shown wavelength (378) nm. The results of the Electron Transition Microscope showed that the biogenic platinum nanoparticles in the current study have mean particle size ranges between (10.83 29.19) nanometers for *Streptomyces fradiae* and *Streptomyces ziwulingensis*, respectively.

The field emission scanning microscope also exhibited the examination of surface morphology, shape, and size, where the average diameter for platinum nanoparticles was calculated; the platinum nanoparticles produced using *Streptomyces ziwulingensis* were spherical with a size in (21-65) nanometers. The particle size distribution was 9.76 nanometers, which confirms that most particles present between 21 and 65 nanometers. At the same time, the FESEM results of platinum nanoparticles biosynthesized using *Streptomyces fradiae* confirm that the particles were spherical with a size between (17-42) nanometers, and the size distribution was 5.55 nanometers. The results of the atomic force microscope showed that platinum nanoparticles have areas ranging in diameter from (11.35– 1442 nanometers) in *Streptomyces ziwulingensis* to the mean diameter of platinum nanoparticles produced using *Streptomyces fradiae* which were (41.66 nanometers), and the Particles size was between (4.89- 227.1 nanometer). The Zeta potential analyses showed that platinum nanoparticles gained negative surface charges at -28.6 mV and -38.1 mV. Fourier Transform Infrared Spectroscopy data shows influential groups that may be involved in the reduction and biosynthesis of platinum

nanoparticles. XRD analyzed the structure and crystalline size of the platinum nanoparticles.

The cytotoxicity of platinum nanoparticles has been tested against A-673 human bone cancer cells and normal cells of the MEF embryo using different concentrations (10, 20, 40, 80) ug/ml; the data showed that these particles showed ability against the revealed cells the highest inhibition ratio was (91% and 87.65%) at 80 µg/ml for *Streptomyces fradiae* and, *Streptomyces ziwlunigenesis* respectively. The statistical analysis results Using Graph Pad Prism software confirmed the existence of essential differences between the inhibitory concentrations of cancer cells at a significant level of >0.05. The antibacterial activity of biosynthesized PtNPs was studied against five species of pathogenic isolate bacteria from different sources. The results showed that PtNPs have a potent effect on Gram-positive and Gram-negative bacteria except for the bacteria of *Pseudomonas aeruginosa*; the highest inhibition zone was against *Staphylococcus aureus* (18.2) mm, whereas the lowest was against *E. coli* (10.1) mm.

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List of Abbreviations

Abbreviation	Key
AFM	**Atomic Force Microscope**
A-673	**Bone Cancer**
CRT	**Chemo-Radiotherapy**
FESEM	**Field Emission Scanning Electronic Microscope**

FTIR	**Fourier transform infrared spectroscopy.**
MEF	**Mice embryo fibroblast cell line**
Nm	**Nanometer**
NCBI	**National Center for Biotechnology Information**
PtNPs	**Platinum nanoparticles**
PMAA	**Polymethyl acrylic acid**
PCR	**Polymerase chain reaction**
ROS	**Reaction Oxygen Species**
RPMI	**Roswell Park Memorial Institute**
SPR	**Surface Plasmon Resonance**
TEM	**Transmission Electron Microscope**
XRD	**X-Ray Diffraction**
XPS	**X-ray photoelectron spectroscopy**

Chapter One

Introduction & Literature Review

1.1Introduction

Recently, inorganic nanoparticles (NPs) have gained vast research interest in the fields of science and technology (Pugazhendhi *et al*., 2018). Nanotechnology generates a diversity of nanoscale materials with a size of about (1–100) nanometers; these nanoscale materials are known as nanoparticle NPs (Nadeem *et al*., 2020). Nanotechnology is a current practice that involves the production, characterization, and application of nanoparticle NPs (Khan *et al*., 2017). 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 (Khan *et al*., 2019).

Nanoparticles (NPs) have gained much attention because of their distinctive physicochemical properties and significant medical applications (Slavin *et al*., 2017). Numerous methods are used to synthesize nanoparticles (NPs), including physical, chemical, and biological.

Studies have concentrated on the biosynthesis of NPs, especially bacterial biosynthesis, due to some properties, including the simplicity of cultivating bacteria and accessible experimental settings, e.g. (pH, temperature) and short generation time (Jang *et al*., 2015). Also, studies have shown that bacterial cells play an essential role in transforming heavy metals into nanoparticles. Additional advantage is their ability to synthesize enormous quantities of long-lasting nanoparticles (Fariq *et al*., 2017).

The metal nanoparticles involved in gold, silver, platinum, and palladium are applied in many applications, such as chemistry, biology, ecology, and medicine (Mallikarjuna, 2019; Jameel, 2020).

Among these nanoparticles, platinum nanoparticles (PtNPs) have generated particular interest due to their unique structural, catalytic, and optical properties, as well as their high surface area and good resistance to corrosion, which makes them a potential candidate for catalysis and biomedical applications (Dong, 2021; Fan,

2021). Platinum nanoparticles have been shown significant success in numerous applications, and they are reckoned to be competent and can serve as drug carriers (Al-Radadi, 2019). Moreover, platinum nanoparticles Pt NPs have various medical uses, including anti-cancer, antioxidant, anti-diabetic, antibacterial, and antifungal applications (Naseer, 2020).

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, and among them, *Streptomyces* genus provides approximately 80% of the industrial antibiotics. 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 nanoparticle's size. Actinomycetes are known to convert metal salts to metal nanoparticles NPs extracellularly and intracellularly. However, the synthesis mechanism of NPs differs depending on the strain type (Bhosale *et al*., 2015; Abd-Elnaby *et al*., 2016).

Cancer is one of the principal causes of death, with a significant increase in its prevalence. The treatment options for cancer are limited, including Chemotherapy, radiation, and surgery. New cancer therapies have faced substantial challenges, for example, the insufficient drug concentration reaching the tumor and the failure to monitor the response to treatment (Ojo, 2021).

Nanotechnology represents a broad field with exponential growth and has the competencies and immense potential in cancer treatment; worldwide studies mainly focus on targeting cancer cells using nano-sized particles; this involves a highly sensitive nano-biomolecule that has attached a transport carrier with attraction for unique surface receptor proteins situated in the cellular wall (Mocan *et al* ., 2015); in this process, a carrier can concentrate the preferred active molecule only in the desired tissue. This aptitude in the nanoparticles to accumulate in enormous

concentrations in targeted tissues or cells may be accomplished by either one or both means of targeting: passive or active. In passive targeting, the nanoparticles (NPs) head directly into the desired cell or tissue through blood flow. Generally, NPs must be between (10-100) nanometers in size to accomplish their function as passive targets and to stay systemically for extended periods. Also, the effects of passive targeting may be enhanced by using drug-loaded NPs to acquire high selectivity to a target tissue or cell; this process is called active targeting (Mocan *et al*., 2017).

There are countless potential applications of green synthesized PtNPs, such as anti-microbial, anti-parasitic, antioxidant, and anti-cancer therapies, which have been testified. NPs are active against various microbial species and exhibit minimal bacterial resistance, which makes them effective as antibacterial 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 (Ahmad *et al*., 2015).

Aim of the study

The present study aimed to investigate the biological activity of platinum nanoparticles synthesized by Actinomycetes bacteria isolated from soil samples by the following steps:

1- Isolation and molecular identification of Actinomycetes isolated from different soil sources.

2- Screening and selecting the best and most potent bacteria for the extracellular synthesis of PtNPs.

3-Physical characterization of the synthesized NPs using Uv-vis spectrophotometer, FTIR, XRD, TEM, FSEM, AFM, and zeta potential analysis.

4- Estimate the anti-cancer activity of biosynthesized PtNPs by Actinomycetes against A- 673 bone cancer cell line.

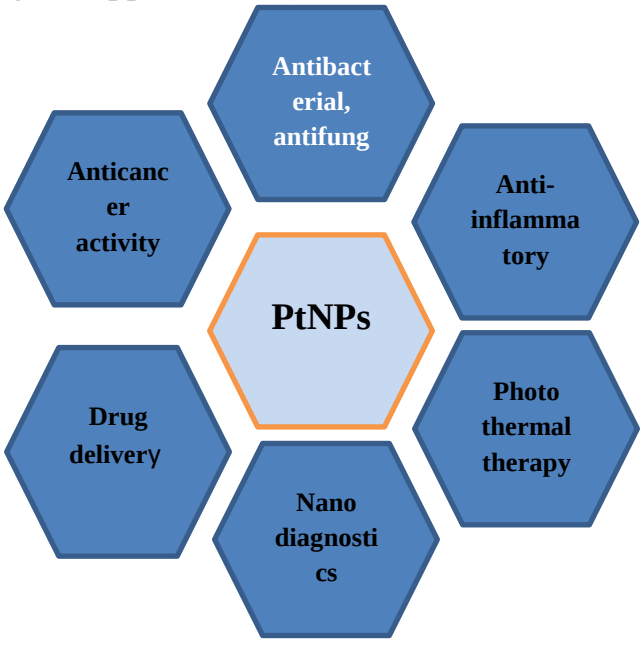
5- Estimate the antibacterial activity of the synthesized PtNPs against pathogenic bacteria.

1.2 Literature review

1.2.1. Nanotechnology

The term nanotechnology was first used in 1974 by Norio Taniguchi (University of Tokyo), which means the ability to engineer materials at the scale of nanometers. Its current meaning is nano-scale materials' production, characterization, and application. Recently, nanotechnology has been considered a central area of scientific research about the preparation, design, and practice of structures, devices, and systems by managing atoms and molecules at the nano-scale levels (Aljuhani *et al*., 2021). It is progressively considered the future of technology (Hoshika *et al.*, 2011).

Nanotechnology has obtained great attentiveness in recent years because it has enormously impacted many fields of science, such as medicine, electronics, energy, etc. Nanoparticles as nano-scale arrangements attract researchers' interest because of their probability of being produced in various sizes and shapes, which can work for advanced biotechnological applications (Jan *et al*., 2021).



Figure(1.1): Multifarious applications of PtNPs

The synthesis of nanoparticles, including physical, chemical, and biological methods, has drawn tremendous attention. Biological methods encompass microorganisms, plant extracts, phages, and various molecules like nucleic acids. These are related to their ability to reduce metal salts and obtain nanomaterials at suitable temperatures and pressures. Chiefly, nanobiotechnology biosynthesis of nanoparticles (NPs) has increased to make novel materials that are ecologically available, cost-effective, and stable NPs with unlimited importance for broader applications, especially in medicine. At present, exciting features of nanomaterials have become prevalent due to their broad spectrum of use in biological and medical fields(Gaidhani *et al*., 2014).

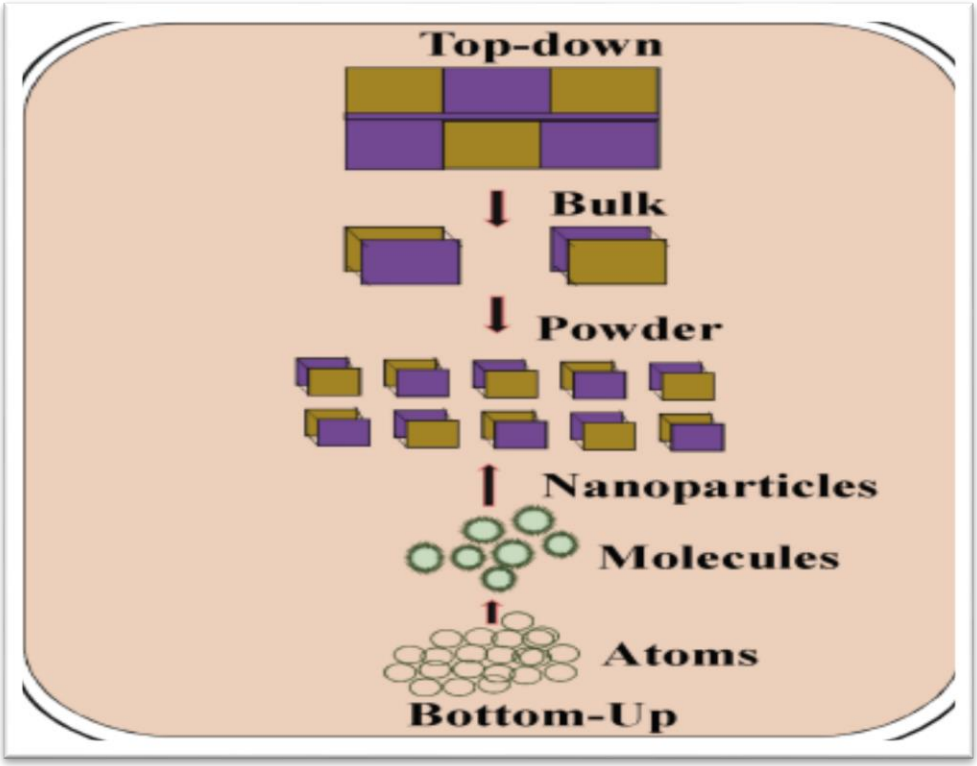
There are two Concepts for the synthesis of nanoparticles**:**

1. Top-down

It includes the use of mechanical methods and physical methods for examples of mechanical method mechanical crushing where method occurs by crushing granules by putting them in a cylindrical container made of alloy Solid, and the crushing of granules occurs as a result of putting granules with balls of greater hardness after emptying the container of air; atmospheric gas is injected instead of an inert gas as prevention of oxidation of the granules. Then, the ball mill runs at high speed, reaching 222 (rpm) to accelerate the large granules grinding, softening, and reduction of their dimensions to be smaller from a hundred nm, in addition to many other physical ways for the nanomaterial's production (Purohit *et al*., 2019).

2. Bottom-up

It is the enlargement of atoms and small molecules by grouping and arranging them, with the presence of electron microscopes that work to examine the internal structure of the materials and enlarge these parts to more than a million Once so that the seeker can see the atoms and molecules (Subramani *et al*., 2019).



Figure(1.2): Synthesis process.

1.2.2. Nanoparticles

Nanoparticles can be defined as substances ranging in size from 1- 100 nm, which is related to their size and may differ from the bulk material. Various metallic nanomaterials are produced using copper, zinc, platinum, magnesium, gold, and silver (Dubchak *et al*., 2010). The nanoparticle biosynthesis of gold, silver, gold–silver mixture, magnetite, platinum, and uranium using bacteria, fungi, Actinomycetes, plants, viruses, and yeast has been mentioned (Narayanan *et al*., 2010).

The nanoparticles of metals have active applications in different areas like electronics, cosmetics, biotechnology, medical technology, energy, pharmaceuticals, drug delivery systems, and food technology fields (Kim *et al*., 2010).

Nanomaterials have distinctive and new properties; when they are less than 100 nms, they appear to have new features and often differ from their known properties in

their natural form. For example, the chemical and physical properties of nanomaterials, such as color, strength, hardness, chemical activity, and thermal properties, showed a diversity and vary between its types and between its constituent materials; the difference in properties is due to two main reasons (Taha, 2014).

1. **Surface area**: the number of atoms present on the superficies of the material increases with the increase in the surface area, and this rises by the reaction of the substance, which makes it highly chemically active. The atoms on the surface of the material are the ones that fall on responsibility for chemical interaction with other atoms because they have unrestricted free electrons within the material, and this property explains the fact that nanomaterials are more active than their normal state, as well as the change in their thermal properties mechanical, electrical and magnetic.

2. **Quantum effec**t: The quantum effect appeared clearly in nanomaterials; because of the infinite dimensions of nanomaterials in Small and close to atomic dimensions, it is no longer subject to the laws of classical physics but to laws of Quantum physics, and this is reflected in the properties of nanomaterials. Two critical factors affect nanoparticles' different properties, which are aggregation and agglomeration, as each affects mechanical resistance, density, electrical and thermal properties, and chemical effectiveness.(Al-Gebory & Menguc , 2018).

1.2.2.1 Classification of Nanoparticles

The nanoparticles were commonly classified into organic, inorganic, and carbon-based:

1. Organic nanoparticles

The organic nanoparticles include dendrimers, micelles, liposomes, ferritin, etc. These nanoparticles are biodegradable and non-toxic; some particles, such as micelles and liposomes, have a hollow core. Also, they are sensitive to thermal and electromagnetic radiation, such as heat and light. These unique characteristics make them an ideal choice for different applications, especially medical ones (Jeyaraj *et al*., 2019).

2. Inorganic nanoparticles

Inorganic nanoparticles represent particles that are made up of carbon. They are further classified into two major categories: metals and metal oxide NPs.(Ealia & Saravanakumar , 2017). The commonly used metals for nanoparticle synthesis are aluminum (Al), cadmium (Cd), cobalt (Co), copper (Cu), gold (Au), iron (Fe), lead (Pb), silver (Ag), platinum(pt) and zinc (Zn). Also, the commonly synthesized metal oxide nanoparticles are Aluminium oxide (Al2O3), Iron oxide (Fe2O3), Magnetite (Fe3O4), Silicon dioxide (SiO2), Titanium oxide (TiO2), Zinc oxide (ZnO). These nanoparticles possess exceptional properties compared to their metal counterparts (Tai *et al*., 2007).

3. Carbon-based

The nanoparticles made entirely of carbon are known as carbon-based. They can be classified into fullerenes, graphene, carbon nanotubes (CNT), carbon nanofibers, carbon black, and sometimes activated carbon in nano size (Ijaz *et al*., 2020).

Nanomaterials are also classified according to the dimensions of the crystalline particles:-

1. Zero – Dimension :

They are nanomaterials that do not have dimensions on the nm scale (Pal *et al*., 2011).

2. One – Dimension :

They are nanomaterials with one dimension on the nm scale, such as the thin films utilized in electronic equipment (Pal *et al*., 2011).

3. Two – Dimension :

Nanomaterials have two dimensions on the nm scale: filters used to separate and filtrate Fine particles such as asbestos fiber (Vincent, 2013).

4. Three – Dimension:

Nanomaterials with three dimensions on the nm scale are essential in many applications, such as electronics (Vincent, 2013).

1.2.3. Synthesis of Nanoparticles

The monodisperse nanoparticle Synthesis with diverse chemical compositions, shapes, and sizes remained a challenge in nanotechnology. Nanoparticles can be produced physically, chemically, or biologically. The chemical and physical methods have accompanied many effects. Instead, the researcher focuses on eco-friendly alternatives to chemical and physical processes, which are the biological techniques of nanoparticle production using microorganisms, fungi, plants, or plant extracts (Ahmad *et al*., 2011); they also have some disadvantages, such as catalysts generation and difficulties in giving of better control of the distribution of size, shape, and crystallinity (Borse *et al*., 2015). These difficulties limit the biological production approaches. However, these can be elucidated by optimizing reaction time,

temperature incubation, pH, metal ions concentration, and the biological material amount.

1.2.4 Green synthesis of nanoparticles

Due to the pollutants emitted by the production of nanomaterials in nature, scientists have resorted to the production of nanoparticles in other ways that are environmentally friendly, and among these methods is the method of biosynthesis by fungi, the technique of biosynthesis by bacteria, and the method of green biosynthesis by plants. As these vital systems work to produce mineral materials by nano-scale size, gold and silver are examples of the production of metallic nanomaterials by biological methods (Sadowski, 2010).

Sherif, 2012) reported the main advantages of using these methods:-

1. It uses environmentally friendly and inexpensive materials
2. It produces nanoparticles with smaller dimensions than the particles produced by chemical or physical methods.
3. Bio-produced nanoparticles have catalytic activity and a larger specific surface area.
4. It does not need stabilizers to prevent the nanoparticles from agglomerating, and even if they aggregate, they do not interfere with each other.
5. The production is high, and the energy consumed is less.

The biosynthesis of nanoparticles in biological ways includes different branches, but the synthesis by microorganisms is a green and eco-friendly technology (Jeyaraj *et al*., 2019; Naseer *et al*., 2020). Various microorganisms, including both eukaryotes and prokaryotes, are utilized to produce metallic NPs (platinum, silver, iron, gold, and metal oxides like zinc oxide, etc.). The microorganisms in this field include bacteria, Actinomycetes, algae, and fungi. Microorganisms like bacteria and fungi regulate the synthesis of metallic nanoparticles due to their tolerance and metal bioaccumulation ability (Fan *et al.*, 2009). The synthesis mechanism of NPs might be

extracellular or intracellular, depending on the nanoparticle's location (Hulakoti *et al*., 2014).

1. Intracellular synthesis of nanoparticles :

This includes carrying ions into microbial cells to synthesize nanoparticles in the existence of enzymes, and by the reduction process, the nanoparticles are synthesized inside the organism cell. The size limitation is probably associated with nanoparticle nucleating within the organisms (Narayanan *et al*., 2010). In this way, the ions of metals are drained into the cell wall through electrostatic interactions. At that time, ion-protein and cofactor interactions inside the cell cause the creation of NPs (Alfryyan *et al*., 2022).

2. Extracellular synthesis of nanoparticles :

Extracellular nanoparticle synthesis has more applications than intracellular production since it is void of unnecessary adjacent cellular constituents in the cell. Generally, fungi are known to synthesize nanoparticles in the extracellular pathway because of their substantial secretory constituents, which are included in the reduction and nanoparticle coating. In this way, ions of metals are reduced by either components of the cell wall or by enzymes, proteins, and organic molecules inside the medium (Wang *et al*., 2022).

1.2.4.1. Biosynthesis of nanoparticles by microorganisms

The utilization of microorganisms is part of the biological approach to NP synthesis. Microorganisms such as bacteria, fungus, yeast, and algae are widely used for NP synthesis due to their ease of culturing, generation time, and ability to grow at ambient Power of hydrogen ion (pH), temperature, and pressure (Fariq *et al*., 2017). For the synthesis of various metal nanoparticles, a wide variety of Bacteria are utilized in the formation of nanoparticles because of their ability to reduce metal ions by special reductase enzymes like Nitro Dependent Reductase or NADH Dependent Reductase (Mishra *et al*., 2015). For example, silver nanoparticles were produced by

E. coli, which was spherical and 31.2 nm in size (Hassan, 2016). *Lactobacillus* bacteria can form silver and gold NPs. It is 13 nm in size (Omidi *et al*., 2014). Another study indicated that AgNPs produced by utilizing Bacillus brevis have newly demonstrated marked antimicrobial properties in *Salmonella typhi* and *Staphylococcus aureus* (Saravanan *et al*., 2018). In addition, platinum nanoparticles can be formed by *Shewanella algae* (Arshad, 2017). Also, the experiments showed that at the lowest cell density with HAuCl4 salt concentration, spherical monodispersed AuNPs in size 19 nm were observed, while in cell number increase that will produce polyhedral AuNPs in size 39 nm. In another study, the platinum, gold, and silver nanoparticles are bio-synthesized using a native bacterium, *Bacillus* (Mollania *et al*., 2024).

Fungi have attracted much research interest in synthesizing bio-metallic nanoparticles because of their ability to carry and bioaccumulate minerals (Siddiqi and Husen, 2016). Fungal detachment is very effective in secreting enzymes outside the cell, so a massive production of enzymes can be achieved by activating the formation of nanoparticles (Longoria *et al*., 2012).The economical ability and simplicity of growing biomass of fungi is another advantage of using fungi in the safe production of metal nanoparticles, as several species can overgrow. In addition, cultivating and preserving fungi in the laboratory is very easy (Moghaddam *et al*., 2015). Fungi can produce metallic nanomaterials via reductase enzymes outside or inside the cell(Duran *et al*., 2007). Many studies registered the biosynthesis of metallic NPs by fungi. Silver nanoparticles have been produced from a wide range of fungal species, such as mushrooms *Penicillium expansum,* which produced spherical silver nanoparticles with a size of 20 nms (Dubey *et al*., 2012). In another study, *P. expunsum* HA2N produced nanoparticles with sizes between 14-25 nms, which showed effectiveness when used as antimicrobials (Ammar and El-Desouky, 2016). This proves that the enzymes of fungi are responsible for reducing metallic compounds in the form of nanoparticles (Zielonka and Klimek, 2017). Experiments

were conducted on *Aspergillus s*p. A group of types of this fungus has led to the production of nanoparticles such as *A.niger.* In another study, platinum nanoparticles(PtNPs) were produced using extracellular fungi using the fungus *Fusarium oxysporum* (Syed & Ahmad, 2012).

The algae is also registered as an organism becoming involved in *Sargassum muticum,* which was utilized to synthesize ZnO nanoparticles and was reported to decrease apoptosis (Sanaeimehr *et al*., 2018). *Sargassum crassifolium,* a macroalgae, was used with seagrass to synthesize AuNPs. *Cystoseira triodes* have succeeded in the biosynthesis of CuONPs of around (7 nm) dimensions and are reported to have enhanced antibacterial and antioxidant properties (Gu *et al*., 2018). Various algae strains, such as *Laminaria japonica, Turbinaria conoides*, and *Sargassum tenerrimum,* have been recorded to produce gold nanoparticles (Swaminathan *et al*., 2011; Ramakrishna *et al*., 2016).

In addition, viruses have appeared as promising organisms in nanoparticle production for biomedical applications due to their biodegradability, biocompatibility, capability of mass production, and affluence of genetic manipulation for desired features. The primary implementation of viral NPs has been in drug delivery, such as vaccines / immune therapeutics. Recently, researchers have found a similar class of substances (virus-like particles) (VLPs) resulting from the protein coat of the viruses (Chung *et al*., 2020) that can synthesize nanoparticles in monodisperse structures. Usually, viral nanoparticle formation includes a generation period in a host body (whether an animal, plant, or bacteria), followed by estimation in-vivo and in vitro (Steinmetz, 2010). These viral nanoparticles have been used in many applications, such as (antimicrobial agents and anticancer agents). Also, it was discovered to advance vaccines for pathogens, e. g (HIV and hepatitis B) (Oh and Han., 2020).

In addition, It is indispensable to consider cautiously handling any viral or bacterial strains that may present pathogenic effects to humans. Thus, to implement

the nano-synthesis of microorganisms on a large scale for viable exploitation, it is also imperative to give to the associated biological safety aspect.

1.2.4.2. Biosynthesis of nanoparticles by plants

Nanoparticles are biosynthesized by the biomass of plants extracted from stems, flowers, leaves, or seeds. The mechanism of nanoparticle production occurs in the presence of metabolites. Nanoactive substances like alkaloids, flavonoids, saponins, steroids, tanning, and nutritious substances act as reducing and stabilizing agents (Khan and Jamil, 2017).

For example, silver nanoparticles were produced from *Rosmarinus officinalis* leaf extract (Sulaiman *et al*., 2013). Zhang *et al*. (2023) showed the green synthesis of platinum nanoparticles(PtNPs) by *Nymphaea tetragona* flower extract using chloroplatinic acid (H_2PtCl_6) as raw material to improve skin health; they also explain that the biosynthesis of metal NPs by living plants, plant extracts and phytochemicals has attracted more and more concern as an easy and valuable alternative method for extracellular biosynthesis of metal NPs. Another study registered the green synthesis of iron nanoparticles using henna plant extract for seed germination and vegetative growth of *Trigonella foenum-graecumL*, in which iron sulfate served as the substrate and henna extract as the reducing agent in the synthesis of FeNPs (Al-Saady *et al*., 2024).

1.2.5 Platinum nanoparticles (PtNPs)

Platinum nanoparticles (PtNPs) are unique because of their large surface area and their various catalytic applications, which have been widely used in the industry, as in medicine and diagnostics, and studied because of their antimicrobial, anticancer, and antioxidant properties in addition to their alloys which shows excellent catalytic properties. Platinum nanoparticles (PtNPs) are mainly used for biomedical applications due to their unique optical and crystalline propreties, which allow them to play the combined role of nanoenzymes, nanocarriers, and nanodiagnostic tools

(Pedone *et al.*, 2017). Generally, platinum Nanoparticles have a wavelength under the critical light wavelength like the other nanoparticles. This makes them transparent and suitable for cosmetics applications and coatings. In addition, nanoparticles of platinum are exceptionally significant in the reduction reactions used and are very important in the industrial creation of fuel cells (Riddin *et al*., 2009).

Different methods were used to produce platinum nanoparticles (PtNPs), Physical, chemical, and biological methods were widespread during the early stages of synthesizing PtNPs (Murphin *et al.*, 2017). These methods offered flexibility in modifying the structure of the NPs to achieve the required morphology and size. The physical method includes high mechanical pressure energy and involves evaporation and condensation to generate PtNPs. This method has many advantages, including that it does not contain toxic chemicals, is pure, and has a uniform size and shape. However, its disadvantages include high cost, high temperature, radiation exposure, and low productivity. Secondly, the chemical synthesis of PtNPs consists of the interaction of atoms and smaller molecules by which the precursor metal ions are transformed into corresponding nanoparticles. Water-soluble precursors are generally used to enhance the reduction of metal ions. The chemical synthesis advantages are cost-effective, thermally stable, high-yielding, controllable, and less dispersed. However, it also has many disadvantages: low purity, toxic chemical usage, and hazardous chemicals to humans and the environment. In addition, using poisonous substances ultimately leads to health and environmental issues (Zhang *et al*., 2016). Therefore, there is a need for "green synthesis" to produce eco-friendly materials (Dhand *et al*., 2015).

However, it is essential to know that studies on the synthesis of platinum nanoparticles are limited compared to the synthesis of gold, silver, and other metal NPs. Limited research has explored the bio-reductive mechanism involved in PtNP synthesis.

Table (1.1): Platinum nanoparticles biosynthesized using various biological entities

Biological Entity	Average size (nm)	Shape	Biomedical Application	References
Quail egg yolk	7–50	Spherical	-	Nadaroglu *et al.*, 2017
Streptomyces species (Gram -positive bacteria))	20–50	Spherical	Anticancer activity against human breast cancer cell lines (MCF-7)	Baskaran *et al*., 2017
Acinetobacter calcoaceticus bacteria	2–3.5	Cuboidal	-	Gaidhani *et al*., 2014
Sheep milk	9	Spherical	-	Gholam-Shabanii *et al,* 2016
Honey	2.2	Nanowires	-	Venu *et al*., 2011
Globular protein bovine serum albumin (BSA)	10–30	Spherical	-	Chen *et al*., 2013
Padina gymnospora (brown algae)	5–50	Truncated octahedral	Bactericidal activity against *Escherichia coli, Lactococcus lactis,* and *Klebsiella pneumoniae*	Ramkumar *et al*., 2017
Padina gymnospora (brown algae)	20–35	Spherical	Anticancer activity against A549 lung carcinoma cells	Shiny *et al*., 2016
Fusarium oxysporum fungus	15–30	Spherical	-	Syed & Ahmad, 2012

1.2.5.1 The bacteriogenic platinum nanoparticles

Numerous bacteria can absorb metal ions on their surface and ultimately reduce them to the corresponding nanoparticles through several mechanisms that may

involve reductases, metallothioneins, and cytochrome enzymes. Many types of bacteria can synthesize platinum nanoparticles (PtNPs), for example, Rhizospheric bacteria, anaerobic sulfate-reducing bacteria, and also photoautotrophic cyanobacteria (Puja and Kumar, 2019; Gautam *et al*., 2020).

Actinomycetes are also reported as active bacteria in the synthesis of PtNPs. Baskaran *et al* (2017) reported the synthesis of platinum nanoparticles using *Streptomycetes sp*. This study mentioned that the NPs synthesized using the members of Actinomycetes present good stability and have enhanced biocidal activities against different pathogens due to proteins involved in the capping of PtNPs. It is considered a well-established, non-toxic, eco-friendly method for synthesizing NPs. Therefore, the use of *Streptomyces sp.* in synthesizing PtNPs with latent anticancer activity looks promising for developing newer nano-antimicrobials by the extracellular method.

Table (1.2): Types of bacteria utilized in the production of platinum nanoparticles

Bacteria	Size	Shape	Localization	References
Acetobacter xylinum	6.3–9.3 nm	Granular	Extracellular and intracellular	Aritonang et al. (2014)
Acinetobacter calcoaceticus PUCM 1011	2–3.5 nm	Cuboidal	intracellular	Gaidhani et al. (2014)
Desulfovibrio alaskensis G20			Extracellular	Capeness et al. (2015)
Desulfovibrio vulgaris Hildenborough (DSM 644)			Extracellular	Martins et al. (2016)
Escherichia coli MC4100	2.3 ± 0.7 nm and 4.5 ± 0.7 nm	Spherical		Attard *et al*., (2012)
Calothrix pulvinata ALCP 745A	3.2 ± 0.3	Well-shaped	Intracellular	Brayner *et al*., (2007)
Pseudomonas aeruginosa SM1	450 nm	Circular disk like	Extracellular	Srivastava and Constanti,(2012)
Shewanella algae	5 nm	Discrete	Intracellular and extracellular	Xu *et a*l., (2019)
Shewanella oneidensis MR-1	3–40	Spherical	Intracellular and extracellular	Xu *et al*., (2019)
Streptomyces sp.	20–50 nm	Spherical	Extracellular	Baskaran *et a*l. (2017)
Sulfate-reducing bacteria	200–1000 nm	Irregular, rectangle and square	Cell-free soluble extract	Riddin *et al*. (2010)
Jeotgalicoccus coquinae ZC15	5.74	Spherical	Extracellular	Eramabadi *et al*. (2020)
Kocuria rosea MN23	5.85	Spherical	Extracellular	Eramabadi *et al*. (2020)

Pseudomonas kunmingensis ADR19	3.95	Spherical	Extracellular	Eramabadi *et al*. (2020)
Pseudomonas putida KT2440	8.06	Spherical	Extracellular	Eramabadi *et al*. (2020)
Psychrobacter faecalis FZC6	2.49	Spherical	Extracellular	Eramabadi *et al*. (2020)
Sporosarcina psychrophila KC19	4.24	Spherical	Extracellular	Eramabadi *et al.* (2020)
Vibrio fischeri NRRL B-11177	3.84	Spherical	Extracellular	Eramabadi *et al*. (2020)

1.2.5.2 The proposed mechanisms of platinum nanoparticle biosynthesis

In general, metals are present in the environment; they can involve cells, crashed membrane structures, inactivate enzymes, and cause toxicity or death of microorganisms in contact with the metal particles. Microorganisms make every effort to avoid metal toxicity by eliminating or accumulating them in forms that are not toxic to the microbes. The formation of NPs by microbes happens as a response to stress, resulting in an adaptive and protective mechanism for the microbe against the toxicity of metals (Ghosh *et al*., 2021). The microorganism removes metals from the cell using enzymatic activity, dissimilatory oxidation, precipitation, or transport by efflux pumps. A suitable proposed mechanism for the synthesis of nanoparticles via microorganisms has not yet been considered since diverse organisms react differently through the synthesis of nanoparticles. Microorganisms create nanoparticles intracellularly and extracellularly. Firstly, in intracellular synthesis, the cell wall plays an essential role in the electrostatic communication of the positive charge of metal ions with the negative charge of the cell wall. That is due to an ion transport system, which transforms the ions inside the cell and the enzymes in the cell, which reduces the metal ions into nanoparticles. Several enzymes, such as reductases, synthases, hydrogenases, and hydrolases, play a vital role in the synthesis and stabilization process. In the extracellular synthesis, the enzymes of

microorganisms secreted in the medium consequently reduce the metal ions into their respective nanoparticles and act as capping agents (Chaudhary *et al*., 2017).

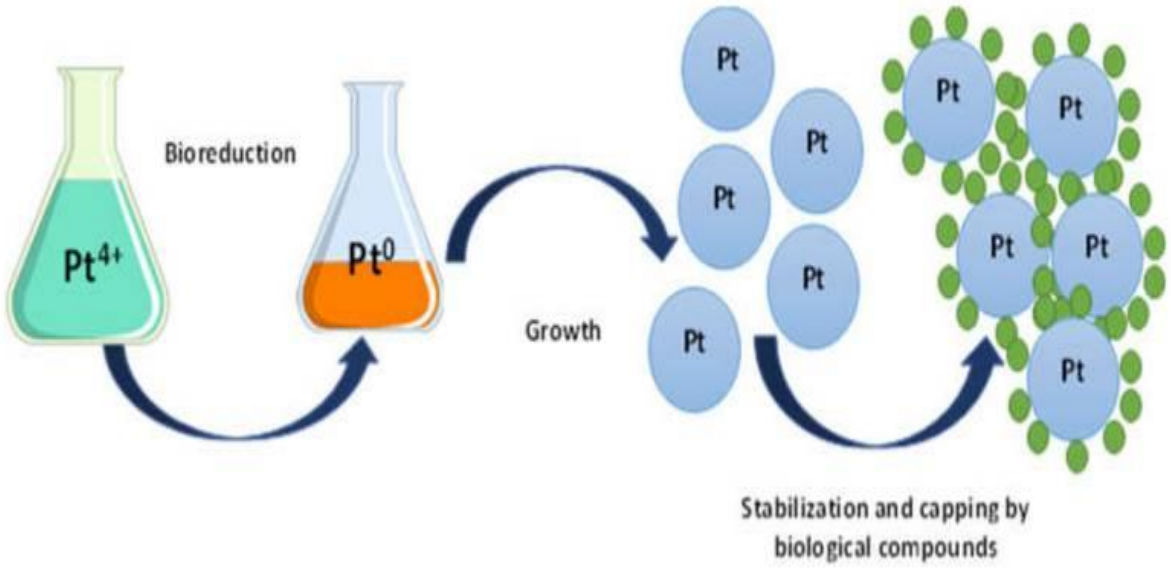
Different mechanisms were reported for the bacterial synthesis of PtNPs in many studies, which must be considerable. Generally, the biosynthesis of PtNPs is based on the biochemical reduction of platinum salts to Pt^0 through redox reactions. The reduction process is implemented via various biopolymers, e.g., proteins, polysaccharides, alcohols, aldehydes, ketones, acids, biologically active substances, and other metabolic products (Aruna & Kavitha, 2021).

Aritonang *et al* .,2014) mentioned that hydrogen gas was used as a reducing agent throughout the formation process of PtNPs using bacterial cellulose (BC) from *Acetobacter xylinum*. In this study, the (K_2PtC_{l4}) in solution goes through several reactions which it is resulting in ($PtC_{l2}(H_2O_2)_2$), and hence hydrogen bonding initiates between the oxygen atom of BC and the hydrogen atom of ($PtC_{l2}(H_2O_2)_2$), PtNPs were reduced in these bacteria on the surface and the inner side of BC membrane. Another study registered the biosynthesis of PtNPs by anaerobic sulfate-reducing bacteria; the formation of NPs was related to the presence of (cytochromes and hydrogenases) enzymes. When the formation begins, they are moved to the outer surface of the cell, forming extracellular PtNPs (Capness *et al*., 2015) Attard *et al* .,2012) showed that *E. coli* MC4100 has numerous hydrogenase enzymes inside the cell which assist in catalyzing the hydrogen into proton and electrons which may facilitate the biogenic creation of PtNPs.

In cyanobacteria, the reduction of metal salts to PtNPs occurs inside the heterocyst and vegetative cells, followed by partial release of PtNPs in the medium. In this bacteria, a reducing enzyme, nitrogenase, exists in the cells and could be responsible for reducing platinum salt PtNPs. Also, cyanobacteria own two classes of reducing enzymes: hydrogenase and nitrogenase. Hydrogenase enzyme reduces hydrogen ions into molecular hydrogen, which may be included in the reaction. Thus, nitrogenase and hydrogenase enzymes can be critical in synthesizing PtNPs in

cyanobacteria. Another study reported the biosynthesis of PtNPs by the participation of two different hydrogenase enzymes in the synthesis of PtNPs. Firstly, the platinum(IV) was reduced to platinum(II) through a two-electron bioreduction via an oxygen-tolerant unique cytoplasmic hydrogenase. Secondly, with the help of another two-electron bioreduction, including an oxygen-sensitive periplasmic hydrogenase enzyme, the platinum(II) ion was reduced to platinum(0) nanoparticles (Riddin *et al*., 2010)

In Actinomycetes, the extracellular synthesis of PtNPs using *Streptomyces sp*. was related to the presence of bacterial chloride reductase enzyme that is usually involved in the nitric acid cycle and is essential in reducing chloride to chlorine. Also, there is Nicotinamide adenine dinucleotide-dependent chloride, which is a reductase enzyme that might play a crucial role in the production of PtNPs where the Pt ions were possibly reduced by an enzymatic metal reduction process (Baskaran *et al*., 2017).



Figure(1.3): The proposed mechanism of platinum nanoparticle synthesis

1.2.6 characterization of nanoparticles

The development of nanotechnology in many studies has led to the necessity of utilizing analysis techniques for nanoparticle characterization and analysis. Several

characterization techniques involve microscopic, spectroscopic, and separation techniques (Gamboa *et al*., 2019). NPs are generally characterized by their surface properties, size distribution, spectroscopic stability, and interactions, so we must look at these main characterization techniques.

1.2.6.1 Morphology and size of the N.P.s (SEM, AFM)

Scanning electron microscopy(SEM) represents a surface imaging technique by which an electron ray interacts with the sample, producing variant signals reflecting surface morphology and atomic composition. (SEM), commonly utilize scattering electrons and secondary electrons emitted through the sample to make the three-dimensional image of the sample analyzed. Though numerous nanoparticles are invisible to the electron microscope, since they do not turn the electron ray enough, the sample preparation involves a coating with a thin metal layer, generating a conductive layer on the sample. This procedure will decrease surface wear, reduce thermal damage, and develop the electron signal needed in the SEM. This technique could be directly obtained based on size, size distribution, and morphology. Nevertheless, this technique has many disadvantages, such as the sample preparation being destructive and one not being sure that the observed image is representative of the sample.

Atomic force microscopy(AFM) is a vital instrument that studies surface measurements and morphology of sensory forces. Generally (AFM) images are gained by identifying the attractive-repulsive forces, usually between the sharp probe and a sample surface. In these techniques, the force is calculated by a laser photodiode system, which distinguishes the difference in voltages on the photodetector output. Like the SEM, the (AFM) gives the size, shape, size

distribution, and aggregation of the NPs (AFM); it has a lesser economic cost than (SEM) and needs less laboratory space. In addition, it is much easier to operate. So, by utilizing these two techniques, each of them will compensate for the other. Also, there is another microscopy technique suitable for the characterization of the shape and size, which is the transmission electron microscopy technique (TEM); in this technique, the image is two-dimensional and formed from two electrons transferred through the sample, the usage of this technique is through their valuably to measure the polymer wall of the nanoparticles.

1.2.6.2 Surface properties and stability

The principal techniques used for the surface properties are zeta potential, photoelectronic X-ray spectroscopy (XPS), energy dispersive spectroscopy (EDS), infrared Fourier transform spectroscopy (FTIR), and Raman. All these techniques reveal the chemical configuration of the surface of the NPs.

Zeta potential analysis is generally used to determine the surface charge and constancy of the colloidal suspension of nanoparticles, which is significant in determining the communications of cell membranes. This analysis is carried out via the electrophoretic movement of charged particles in an applied electrical potential.

Energy dispersive X-ray spectroscopy (EDS) is a chemical analysis technique that usually works with electronic microscopy tools like SEM. Its work is based on the "fingerprint" energies of the X-rays released by the samples. In addition, it is possible to achieve a general sample mapping by performing the X-ray spectra in scan mode.

Infrared spectroscopy or Fourier transform (FT-IR) and Raman spectroscopy are two forms of vibratory spectroscopy that are beneficial for examining the structural properties of nanoparticles. Infrared spectroscopy is generally used to determine the characteristics of diverse nanoparticles involving metal and carbon nanomaterials besides core nanoparticles (Merck, 2020).

1.2.7. Actinomycetes

Actinomycetes are Gram-positive bacteria, filamentous in shape, belonging to the phylum Actinobacteria, which signifies one of the largest taxonomic units among the 18 significant lineages presently recognized inside the Domain Bacteria (Ventura *et al*., 2007). Actinobacteria are characterized by a complex life cycle and broadly distributed in both aquatic and terrestrial environments, chiefly in soil, where they play a vital role in recycling biomaterials by decomposition process on the complex mixtures of polymers in dead animals, plants, and fungal resources. Actinomycetes also have a critical and vital role in the soil through biodegradation and humus formation processes, such as they recycle the nutrients related to recalcitrant polymers, for instance, chitin, keratin, and lignocelluloses (Stach and Bull, 2005) this produces some volatile substances like geosmin, which is responsible for the characteristic "wet earth odor" and show various physiological and metabolic properties, such as the manufacture of extracellular enzymes.

The studies showed that the secondary metabolites produced by microorganisms are reported to be near 23,000, of which Actinomycetes produce 10,000. Therefore, they represent 45% of all bioactive microbial metabolites. Between the Actinomycetes, around 7,600 compounds are made by *Streptomyces* species (Berdy, 2005). Some of these secondary metabolites are effective antibiotics, and members of this group are also registered as producers of useful anti-tumor drugs such as anthracyclines**.**

Actinomycetes are characterized by the formation of mycelium on solid media and the presence of spores, which could be with different spore surfaces and high (Guanine + Cytosine) content of DNA dependent on morphological and chemical principles. Actinomycetes have been grouped into diverse genera, from which *Streptomyces* is the most common genera of the order Actinomycetales because of its unlimited importance in medical science, ecology, industry, and biotechnology (Karuppiah and Mustaffa, 2013).

Actinomycetes are heterotropic in their natural nutrition; many are severe saprophytes, while some are parasitic or mutualistic in associations with animals and plants. These MOs are usually believed to play a role in recycling natural nutrients. They are aerobic in their requirement for oxygen, and some are anaerobic, like *Actinomyces*.

1.2.7.1. Actinomycetes distribution

Actinomycetes are up to survive in different habitats and are generally distributed in natural ecosystems. Actinomycetes populations have been recognized as soil inhabitants. The researchers focus on screening new Actinomycetes that have not been discovered because it was stated that only 10% of Actinomycetes was isolated from nature; in the soil, the distribution of Actinomycetes show diversity, some groups of Actinomycetes are stable in bulk soil and others in rhizosphere plants, where rhizospheric Streptomycetes can protect plant roots via inhibiting the progress of fungal pathogen (Gonzalez *et al*., 2009). Also, there are Actinmycorrhizal plants, characterized by nitrogen fixation nodules in their roots that have been combined with Actinomycetes presence (*Frankia* Dwary *et al*., 2011).

Hypersaline soil represents a habitat for some Actinomycetes. These habitats are identified as typical extreme environments that contain saline lakes and saline soils. Examples of Actinomycetes genera isolated from hypersaline soils were *Streptomyces alboflavus*, *Nocardia* sp., *Micromonospora* sp., and *Streptomyces griseoflavus*. Actinomycetes are also distributed in freshwater habitats. It was known in recent years that the value of this habitat was a source of Actinomycetes, in which

the genus Streptomyces existed dominantly in the river water. In contrast, the genus *Micromonospora* was dominant in the river's sediments.

It must be known that the distribution of Actinomycetes in the aquatic environments has been to a great extent, undiscovered, and the presence of marine Actinomycetes in the oceans remains a mystery. (Murti *et al*., 2010).Marine Actinomycetes are very significant microorganisms because of their essential role in biotechnological and biological applications. Actinomycetes are found and isolated in air. Spores can be in the air, and it was detected that air contains diverse types of spores. Some research has stated that spores of airborne Actinomycetes, such as Nocardia sp., are responsible for some antimicrobial production (Asan *et al*., 2004).

Some Actinomycetes are found in infrequent marine conditions, for instance, in the reservoirs of marine gas hydrate in deep-sea and organic aggregates, representing the main components of the microbial communities in this habitat. Also, some actinomycetes are isolated from marine organic aggregates found in the Wadden Sea; these exhibit high antagonistic activity inside this habitat. These Actinobacteria produced valuable compounds that could used in the pharmaceutical industry (Lam, 2006). It was stated that marine Actinomycetes provide a revelation of novel classes of therapeutics that give the medical sciences the expecting through the following several years that could fight against drug-resistant infectious diseases (Jakubiec *et al*., 2018).

1.2.7.2. Actinomycetes Identification

1.2.7.2.1. Molecular Approach

The most significant approach to identification is the study of nucleic acids. Molecular science, which has both classification and identification, has its derivation in the early supermolecule hybridizing studies, and later, the studies on the Sequences of 16S rDNA can provide actinomycetologists with a phylogenetic tree that enables the researchers of evolution in Actinomycetes to provides the principles for identification. The analysis of the 16S rDNA begins through analytic DNA and

increasing the gene coding for 16S rRNA using the enzyme chain reaction (Valli *et al*., 2012).

1.2.7.2.2.Classical approach

1. **Morphological characterization** The isolates and colony morphology will noted concerning many essential features, such as pigment production, the presence or absence of aerial and substrate mycelium, and the nature of the colony (Daquioag *et al.*, 2021).
2. **Biochemical Characterization**. The Gram-positive isolates were chosen for biochemical features; the biochemical tests include catalase, urea hydrolysis, citrate utilization, indole, methyl red, and VogesProskauer (Mohamed *et al*., 2021).

1.2.8 Medical Applications of Nanoparticles

1.2.8.1. Antimicrobial activity

Several papers on using metallic nanoparticles for antibacterial activity have shown advantageous results (Singh *et al*., 2018; Jayabalan *et al*., 2019). One of the most essential features of nanoparticles is their large surface area in contrast to their small size, increasing their interaction with many other molecules. These unique features have generated interest in different applications, such as biological sensors, drug development stimulants, cancer imaging, and pharmaceutical production techniques (Gahlawat *et al*., 2016; Khan *et al*., 2019). The researchers have tried to understand the mechanisms behind the antimicrobial activity(Dakal *et al*.,2016) and (Susanti *et al* .,2022) represented many mechanisms these are:

1. Linking with the cell membrane directly.
2. Generation of reactive oxygen species (ROS).
3. Weakening and alteration in permeability of the cell membrane.
4. Variation of signal transduction pathways.

Many studies have improved the role of platinum nanoparticles in antibacterial activity, especially in the recent overuse of antibiotics, which has led to the growing prevalence of antibiotic-resistant bacteria. Also, with the increasing prevalence of bacterial infections in hospitals, especially in immunocompromised patients, such as those with cancer, there remain limited treatment options to combat these bacteria (Ventola, 2015). (Taha *et al* .,2023) showed the screening of platinum nanoparticles produced by *F. carica* as an antifungal and antibacterial agent against *Candida* and *Aspergillus* species as well as Gram-positive bacteria like *Staphylococcus aureus* and Gram-negative *Acinetobacter* sp, respectively the synthesized PtNPs demonstrated inhibition zones against fungal and bacterial species. The active role of PtNPs in antifungal and antibacterial activities may indicate the presence of a well-regulated nanomaterials system for biomedical applications. Another study showed the PtNPs synthesized using *Atriplex halimus* leaves as an antibacterial agent against Gram-negative and Gram-positive bacteria, which was determined using the inhibition zone. The bacterial strains used in this study are E.coli, Klebsiella pneumonia, Gram-negative bacteria, and Gram-positive bacteria such as *Bacillus subtilis* and *Staphylococcus aureus*. The researchers in this study explained the result depending on that PtNPs may have antibacterial characteristics by changing the shape of the cell membrane (bacterial cell) and inhibiting normal budding because of the loss of membrane integrity (Eltaweil *et al*., 2022).

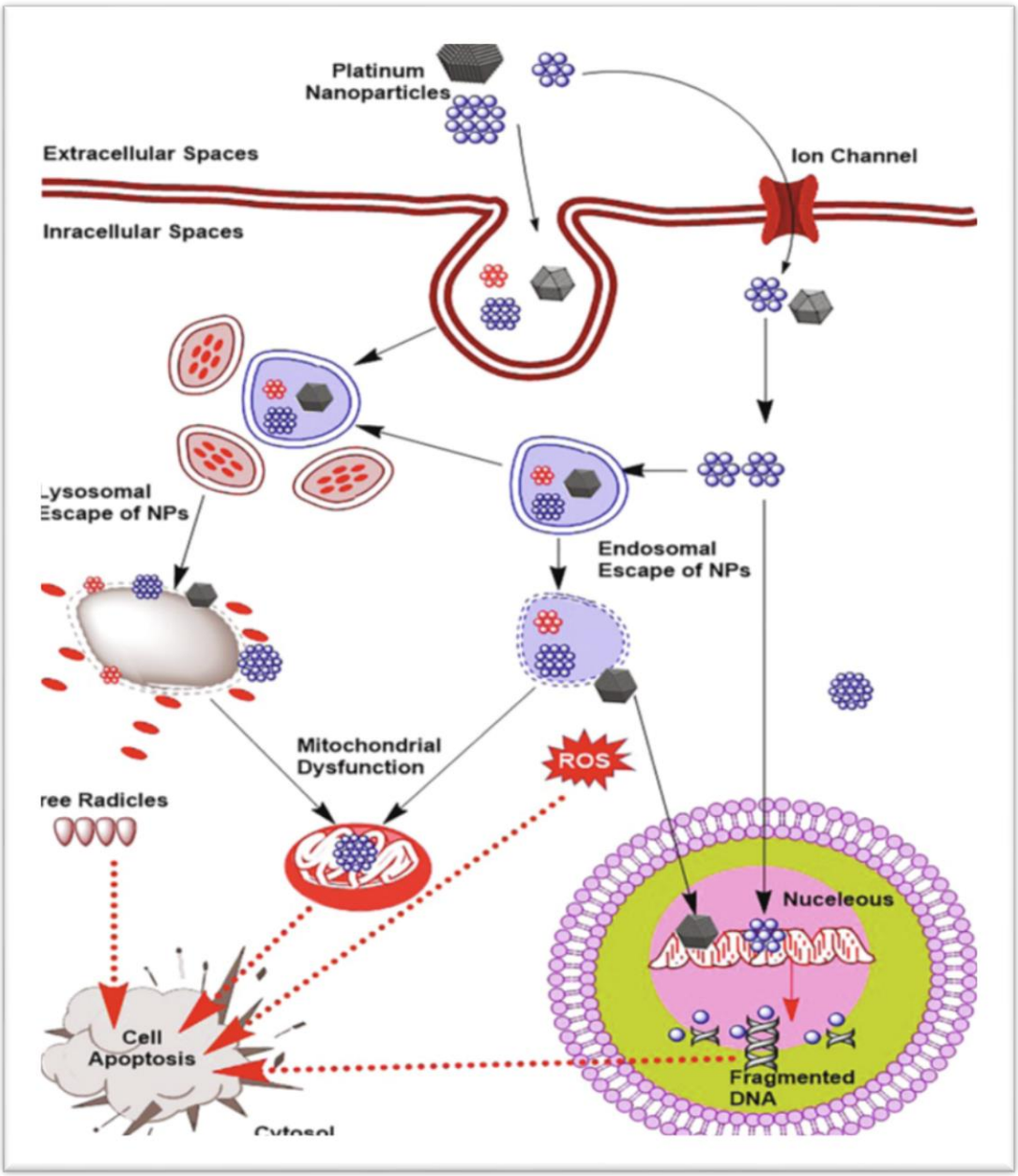
1.2.8.3. Anticancer activity

Cancer is a terrible global disease and represents a primary cause of death worldwide; cancer led to the mortality of 8.8 million people in 2015 (Vos *et al*., 2017) and nearly 10 million people in 2020 (Sung *et al*., 2021). This term is commonly used to describe a collection of diseases characterized by the abnormal and uncontrolled growth of cells. The development of cancer can be summarized into four distinct stages: cancer initiation, tumor dissemination, metastasis to distant organs, and resistance to chemotherapy.

The treatment of cancer includes surgery, radiotherapy, immunotherapy, and chemotherapy. Chemotherapy is used in a high percentage of cancer cases as standard treatment. However, it has some disadvantages, such as the low effectiveness of targeted drug delivery to cancer cells and its effect on healthy cells, which leads to side effects such as fatigue, hair loss, nausea, vomiting, and inflammation. Higher doses are used to achieve the desired drug concentration in cancer cells, which increases side effects.

Nanoparticles have been studied for their aptitude to induce autophagy and promote cell death. Also, biological metallic N.P.s are cytotoxic agents that can fight various cancers (Patil and Chandrasekaran, 2020). (Pallavi *et al*.,2022) discover that AgNPs created by *Streptomyces hirsutus* have toxic effects on human lung cancer.

Several reports in the research describe the mode of action of NPs towards cancer cells (Sampath *et al*., 2022). However, the exact mechanism of cytotoxic effects remains unknown. The interaction between NPs and cancer cells is believed to occur in different ways; these may include electrostatic interaction between the cell's surface and NPs, nanoparticles surrounded by cell receptors, and then ingested by endocytosis. Nanoparticles are well known for their aptitude to produce ROS inside the cell, which can result in protein oxidation, mitochondrial dysfunction, DNA damage, and cell death (Patil and Kim, 2017; Sampath *et al*., 2022).



Figure(1.4): Sketch of PtNPs inducing cytotoxicity in cancer cell lines.

1.2.8.3.1. Bone cancer

A malignant bone tumor is known as an aggressive and unpredictable disease that tends to spread, which should lead to treatment with surgical resection to avoid malignancy, according to the newest Guidelines of the National Comprehensive Cancer Network (NCCN). Treatment aims to prevent bone damage adjacent to the affected joint. Still, widespread excision of the tumor will go out of the bone, causing defects challenging to repair)Weber et al., 2008). Commonly, autogenous or allogenic bones restore the function of bone, but later, unfortunately, they lose the resources or could be at risk of immune rejection (Roberts and Rosenbaum, 2012). Besides, the residual tumor cells that are not entirely removed raise the risk of tumor return (Leary et al., 2013); it's essential to know that the bone substitutes presently in clinical use still lack enough bioactivity to increase bone regeneration (Lin et al., 2019) In addition, the absence of anti-tumor function makes the substitutes unable of killing tumor cells which may stay after clinical surgery. Then, the chance of activation of tumor cells rises (Dang *et al*., 2018).

Consequently, there is a vital need for an artificial, highly bioactive bone substitute that attains syncretic bone regeneration and therapy for bone tumor treatment (Foroughi *et al*., 2020).

Chapter Two

Materials & Methods

2. Materials and Methods
2.1. Materials
2.1.1. Equipment and Apparatus

Table (2.1): Apparatus Used During the Study Period with The Name of the Manufacturer and the Country of Origin

NO	The Apparatus	Company/origin
1	Autoclave	**Hirayama/Japan**
2	Incubator	**Human Lab/Korea**
3	Microwave	**Shownic/Korea**
4	Vortex	**Labco/Japan**
5	Refrigerator	**Vistal/Poland**
6	UV-visible spectroscopy	**Shimadzu/Japan**
7	Electrophoresis	**Consort/Belgium**
8	Water distillatory	**GFR/Germany**
9	Centrifuge	**Hittich/Germany**
10	Hot plate with a magnetic stirrer	**Heidolph/Germany**
11	Gel system documentation	**Vilberlourmat/France**
12	Shaking Incubator	**Zenith Lab/China**
13	Water path	**Memmert/Germany**
14	Oven	**Memmert/Germany**
15	Biosafety	**Lab Tech/France**
16	Light Microscopy	**Olympus/Japan**
17	Sensitive Balance	**Sartorius/Germany**
18	Thermo cycler	**Prime/UK**

19	Vaccum pump	**Knflaboport/USA**
20	X-Ray diffraction	**Phillips/Holland**
21	Atomic force microscope(AFM)	**NT-MDT/ Russia**
22	CO_2 incubator	**Cypress Diagnostics/Belgium**
23	Laminar flow hood	**K & K Scientific Supplier/Korea**

Table (2.2) Equipment Used During the Study Period with the name of the Manufacturer and the Country of Origin

NO	The Equipment	Company/Origin
1	Petri Dishes	**Bio zek Medical/Holland**
2	Test tube	**ALS/Canada**
3	Filter paper	**Watman No.1/UK**
4	Epindroff	**Bio neer/Korea**
5	Screw cap bottles	**Pyrex/England**
6	Micropipettes 0.5-10µL, 10-100µL, 100-1000µL	**Dragon/China**
7	Gloves	**Broche/ Malaysia**
8	Slides and cover slides	**Superstar/India**
9	Beaker	**General/USA**
10	Flask	**Iso Lab/Germany**
11	Disposable Syringes	**Superstar/India**
12	Standard wire loop	**Himedia/India**
13	Benzen burner	**Gallenkamp/ England**
14	Cell culture plate	**Thermo Fisher Scientific/USA**

2.1.2 Media and Chemicals

2.1.2.1 Media that Prepared in Lab. Table (2.3)

NO	Culture media	Abbreviation	Composition g/L of DW
1	Starch-Casein-Nitrite Agar (Bernard, 2007)	SCN Agar	Starch Casein Potassium Nitrate Potassium Monohydrogen Phosphate Magnesium Phosphate Calcium Carbonate Agar
2	Yeast Extract Glucose Agar ((Bernard, 2007	YEG Agar	Glucose Yeast Extract Potassium Nitrate Potassium Monohydrogen Phosphate Agar
3	Malt extract Glucose Yeast Extract Peptone Broth (Dayma (et al.,2019	MGYP broth	Malt extract Yeast extract Glucose Peptone

2.1.2.2 Culture Media (Ready to Use)

Table(2.4): Culture Media

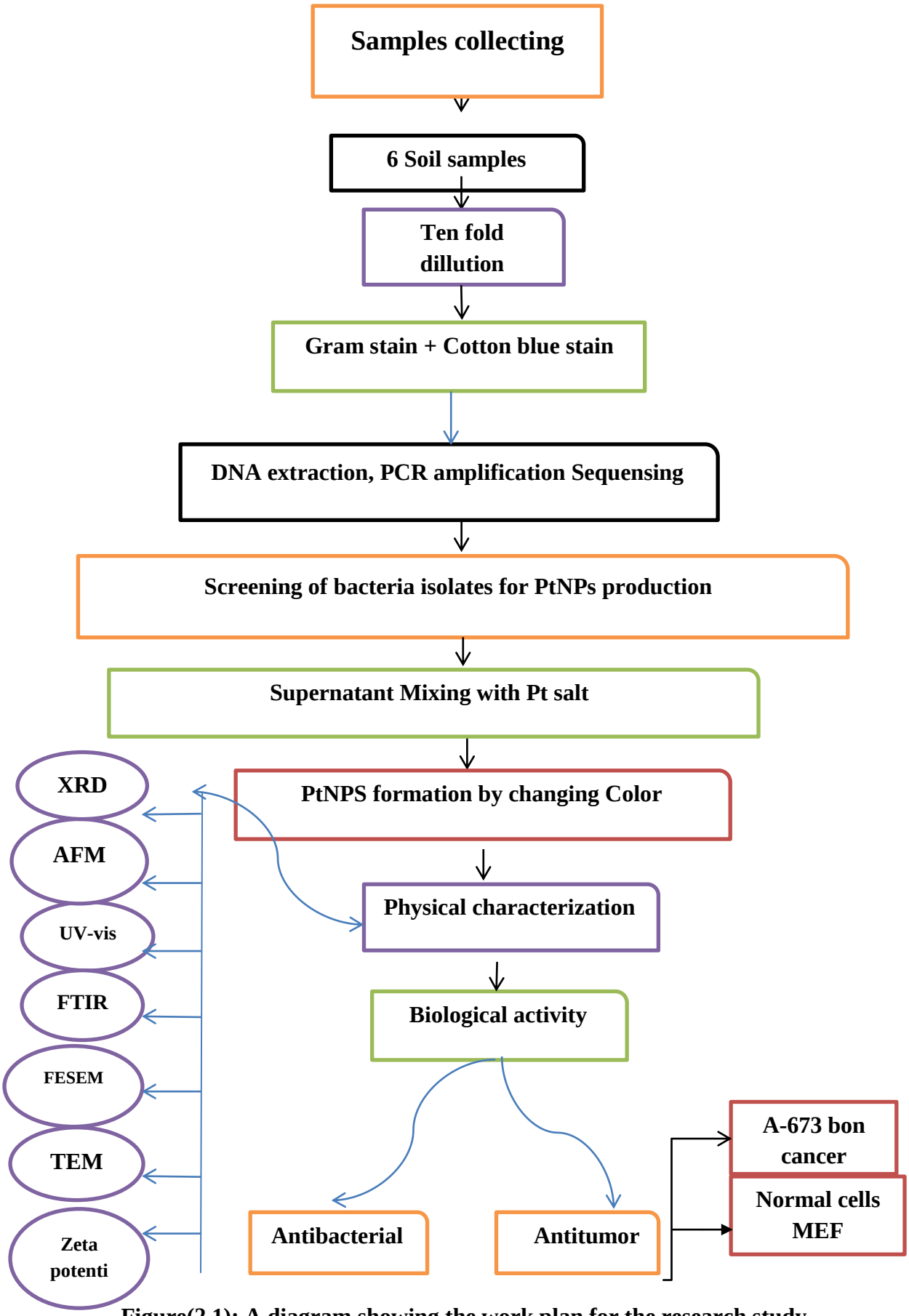
NO	Culture Media	Company/Origin	/Amount g L of DW
1	Nutrient Agar	Himedia/India	**28 g**
2	Mueller Hinton Agar	Michigan/USA	**38 g**

All media was sterilized at 121oC in an autoclave for 15 min.

2.1.2.3 Biological and chemical materials

Table (2.5): All biological and chemical materials used in the study with the Name of the Company Manufacturer and Country of Origin

N0	Chemicals	Company	Origin
1	Agar	KR	**Chile**
2	Absolute ethanol	RBL	**Spain**
3	Primers	Bioneer	**Korea**
4	Deionization water	Ajax	**Australia**
5	Agarose	Bio basic	**Canada**
6	Ethanol 70%	BDH	**England**
7	Cycloheximide	Himedia	**India**
8	Chloroplatinic acid hydrate	Sigma Aldrich	**USA**
9	DMSO	Santa Cruz	**USA**
10	Gentamicin Disks	Liofilchem s.r.i	**Italy**



Figure(2.1): A diagram showing the work plan for the research study

2.2 Methods

2.2.1 Soil Samples Collecting

Thirty Soil samples were gathered from several locations, including sugar cane fields and gardens, from(10 October 2022 to 24 December 2022). Samples were taken from a depth of (8-10cm). 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.

2.2.1.1 Soil Samples Drying

In this study, the soil samples were dried for three hours in a hot air oven at 60C˚ in the biology laboratory/college of Science / Misan University. This study aimed to reduce the number of bacteria in the soil, except Actinomycetes. Actinomycetes produce spores in the soil, which germinate and develop in the media; this is one of many methods for selectively isolating Actinomycetes from soil samples. Then, the dried samples were transferred to tubes, reacted with 0.2 g of CaCo3 (Sapcota et al., 2020), and kept at 4C^{o}.

2.2.1.2 Isolation Media

In this study, Actinomycetes were isolated using a starch-casein-nitrate agar media(SCN) as an isolation medium; SCN agar medium was placed in the flask, which was then plugged with cotton, and the pH was checked before being sterilized in121C˚ autoclave for 15 minutes. The medium is allowed to cool to roughly 50C˚, then Cycloheximide (1ml) is added at a concentration of (100ug/ml) before being poured into the plates.

2.2.1.3 Dilution of Soil Samples

Four sterile test tubes with (9 ml) of sterile distilled water were labeled as 1, 2, 3, and 4. One gram of dried soil sample was added into test tube one and mixed well. One ml of soil solution from tube one was transferred to tube 2 using an I ml micropipette and mixed well. This serial dilution was done up to tube 4. 1 ml solution

was discarded from tube 4. Thus, the concentrations of 10-1,10-2,10-3 and 10-4 g/ml were obtained (Hayakawa, 2008)

2.2.1.4 Dilution Plating

Four Petri dishes containing isolation media were labeled 1,2,3, and 4 for each dilution's test. Starting with tube 4, transfer 0.1ml of the solution to the Petri plate that had been labeled. Each soil solution was distributed on the agar medium immediately after transfer using a sterilized glass spreader. The plates were then incubated for five days at 30C˚**.**

2.2.1.5 Pure Culture Isolation

The selective medium (YEGA) is heated to dissolve the ingredients. After that, it was autoclaved for fifteen minutes at 121C˚. The medium was then allowed to cool to roughly 50C˚, and(1ml) of Cycloheximide (100µg/ml) was added before being poured over the plates, then the medium was poured onto the plates. Then, the isolated colonies were transferred to a selective medium and incubation. On one plate of transfer media, one colony was transferred. The plates were then incubated for 7-14 days at 30C˚.

2.2.1.7 Morphological Identification

The plates were examined after the incubation period; colonies were reviewed to see the morphological features, for example, if they are tiny, opaque, compact, pigmented, or dull. Under a light microscope, the colonies were examined for colonial morphology and to distinguish other bacteria from Actinomycetes.

2.2.1.8 Pure Cultures Maintenance and Preservation

One of the most commonly utilized preservation techniques is the slant technique. After preparing the YEGA medium (transfer medium) and Nutrient agar, they were transferred to test tubes in volumes of about 5ml, and the tubes were kept on a slanted tray. The Pure cultures were transferred to test tube slants and incubated

at 30C˚ for five days. After that, if the growth was acceptable, they were covered with Parafilm and stored at 4C˚ in the refrigerator for future use (Hassan, 2018).

2.2.2 Molecular identification of the isolates

2.2.2.1 Extraction of bacterial genomic DNA

The genomic DNA was extracted from all bacterial isolates grown on cultures media plates using Presto™ Mini g DNA bacteria kit according to the manufacturer's instructions. The genomic DNA was stored at -20C˚.

The steps were as follows:-

1. Add 1 ml of active bacteria to the Eppendorf tube and place it in the centrifuge at 14,000 rpm.

Add 180 microliters of buffer G to the tube. 2.

3. Add 20 microliters of proteolytic enzyme type Proteinase K, then add deionized distilled water and mix it using a vortex device.

4. Place it in a water bath at 60 degrees Celsius for 10 minutes and shake it every three minutes during the water bath.

5. Add 200 microliters of GB buffer solution and place it in the Vortex shaker for 10 seconds

6. Place it again in a water bath At 70C˚ for 10 minutes

7. Add 200 microliters of 99% concentrated ethanol.

8. Use GD Columm filters in 2 ml for each sample of the mixture after the mixture is transferred to sterile tubes called collection tubes and placed in a centrifuge at 15,000 revolutions for 2 minutes

9. Add 400 microliters of W1 buffer to the GD Colum, then put it in the centrifuge at 15,000 revolutions for 30 seconds to get rid of the filtrate

10. Add 600 microliters of Wash Buffer solution, then place it in a centrifuge at 15,000 RPM (GD) into a tube for 30 seconds

11. Add 100 microliters of the previously prepared Buffer Elution solution at room temperature 25 degrees Celsius for 3 minutes and then place it in the centrifuge at 15,000 revolutions for 30 seconds

12. The last step is to remove the GD filter and save the covered tube containing the bacterial DNA to be isolated

2.2.2.2 Gel Electrophoresis to Confirmation of the presence of extracted DNA

1. The agarose solution was prepared by dissolving 0.4g of agarose powder in 50 ml TBE buffer (1X) and gently mixed.

2. The solution was heated to boiling point in the microwave oven for 3 minutes and waited until the solution became clear

3. After the mixture had cooled to 50-60 C˚, two µl of ethidium bromide was added and mixed gently.

4. To make the wells, the comb was placed at one end of the prepared gel box; after that, the agarose solution containing ethidium bromide was poured into it and left until solidification

5. Then the comb was carefully removed from the box, and the gel was

transferred to the electrophoresis tank, where the diluted buffer solution was poured into it to cover the gel's surface approximately 3 to 4 mm.

6. 3 µl of template DNA and two µl of the bromophenol blue dye were mixed and loaded into agarose gel wells using a micropipette

7. Then, the electrodes were linked in their specified positions, and the running was carried out for 30 min at 120 mA 80 V. Finally, gel electrophoresis results were visualized by a gel documentation system (Lee *et al*., 2012).

2.2.3 Amplification of 16S rDNA gene

All the isolated bacteria were identified by 16S rDNA gene (1500 bp) Amplification of 16S rDNA using a thermocycler.

2.2.3.1 Preparation of primers

According to the instructions of the primer synthesizer company, the primers (originally lyophilized) were dissolved in the free ddH_2O to obtain a final concentration of 100 µM/µl, which served as a stock solution that was stored at -20 C˚. A concentration of 10 µM/µl was prepared from the stock primers.

Table (2.6) Primers sequences for amplification of 16r DNA gene

Target	gene	Sequence (5'-3')	Tam (C˚)	Product size	Reference
16S	143	GATGACGTCAAATCATCATGC	56	900 bp	Miyoshi, *et al.*, 2005
rDNA	DG74	AGGAGGTGATCCAGCCGCA			

GoTaq® 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® 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.

Table(2.7) Preparation of PCR solutions

Components	Concentration	Volume (50 µl)
2X PCR *Taq* Master Mix	1X	25 µl
Forward primer	10 µM/µl	4 µl
Reverse primer	10 µM/µl	4 µl
ddH_2O	-	13 µl
DNA	40 ng	4 µl

Table(2.8) PCR program for amplification the 16SrDNA gene

Phase	Temperature(C˚)	Time	N0 of Cycles
Initial denaturation	94C˚	5 min	1
Denaturation	94C˚	30 sec.	35
Annealing	56C˚	30 sec.	
Extension	72C˚	1 min	
Final extension	72C˚	5 min	1

2.2.3.2 Electrophoresis of PCR Products

The amplified DNA fragments were separated using an electrophoresis apparatus**,** and about five µl of each PCR product was inserted into the middle of the correspondence hole in the 1% gel. About five µl of Safe-Green 100bp Opti-DNA

Marker was added to the first hole in the lines of the gel to serve as a marker for measuring the size of the PCR products; the electrophoresis system was set as follows: 60 Volt, constant current, 45 min. Time Finally, the gel was transferred into the UVP system to visualize the PCR products under a 320nm UV light source.

2.2.4 Sequencing of 16S rDNA gene

The samples were labeled and sent to a Macrogen biotechnology company (South Korea). The same company purified the PCR product and analyzed the sequence of forward and reverse 16S rRNA primers. The sequence results were compared with the ready gene sequences at the National Center for Biotechnology Information (NCBI).

2.2.5 The biosynthesis of platinum nanoparticles

2.2.5.1 The biosynthesis of PtNPs by bacteria isolates

Platinum nanoparticles were prepared following the method described (Borse *et al*., 2015). Bacterial strains that have been isolated, purified, labeled, and cultured in MGYP broth and nutrient broth (pH 7) at 29C˚ for seven days with constant shaking on a shaker incubator (150rpm). After the incubator, it was filtered through Whatman filter paper (No.1) and centrifuge (14000rpm), by which the biomass was filtered to separate it and obtain the supernatant. The solutions used to synthesize PtNPs are made by mixing (v/v)100 ml of supernatant with 100 ml of chloroplatinic acid hydrate (2 mM). 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 chloroplatinic acid hydrate; in both ways, the solutions were kept on a rotary shaker (150rpm) at 29C˚ 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 (Jameel *et al*., 2020).

The mixture was separated in a centrifuge for 20 minutes at 8000 rpm, the supernatant was replaced with DDW centrifuge three times, and the pellets were dried at 45C˚ for one day; the powder is retained in vials for additional testing (Aldujaili *et al.*,2020) with minor modification.

2.2.5.2 The physical Characterization of the synthesized platinum nanoparticles

The main factors studied in the physical characterization of nanomaterials are size and shape; also, we can measure size distribution, degree of aggregation, surface charge, surface area, and organic bonds on the surface of the particles, which may influence other properties and their potential applications. Furthermore, their crystal structure and chemical composition are investigated as a first step after nanoparticle synthesis (Verma and Maheshwari,2018).

2.2.5.2.1. 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 (Eltaweil *et al*., 2022).

2.2.5.2.2 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. Which facilitates the biosynthesis process. The samples (in powdered form) were measured using the FTIR spectrometer in the 400–4000 cm^{-1} (Huq and Akter, 2021).

2.2.5.2.3 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. X'pert Pro X-ray measured the samples diffract meter, and the diffraction pattern of the powdered form of biogenic NPs was recorded from 10º to 80º (2 theta), with a step size of 0.050º, 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 (Qais *et al*., 2019).

2.2.5.2.4 Transmission electron microscopy (TEM)

TEM was used to analyze synthesized nanoparticles' size, shape, and distribution (PtNPs).

2.2.5.2.5 Scanning electron microscopy (SEM)

The SEM was used to observe the morphology traits of the platinum NPs synthesized by bacteria. The process involves adding a small drop of NPs suspensions to the slide and drying it before SEM analyzes it. The microscope runs at different magnifications (Saleh and Alwan, 2020).

2.2.5.2.6 Atomic force microscopy (AFM)

The AFM device characterized 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 (Kotakadi *et al*., 2016).

2.2.5.2.7 Zeta Potential Analysis

A zeta potential analyzer instrument was used, and the zeta potential method was used to measure the stability of the platinum NPs. The samples were centrifuged, and the NPs were measured between -200 and +200 mV at 25.2 C˚ (holder temperature) (Pallavi *et al*., 2022).

2.2.6 Applications of platinum nanoparticles

2.2.6.1 Antibacterial activity test

The antibacterial activity of the manufactured PtNPs was examined using the method (agar disc diffusion) against several pathogenic bacteria isolates from clinical specimens (Thapa, 2017). The specimens were from patients in AL Sadr Hospital in Misan, Iraq. Five isolates 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. The swap was used to culture the specimens on blood agar and MacConkey agar at 37 C˚ for one-day incubation in the hospital.

2.2.6.1.1 The Preparation of PtNPs Discs

1. In the test tube, 10μg of the synthesized PtNPs was put.

2. 1 ml of DMSO was added to the test tube.

3. A suspension was made by shaking the test tube very well.

4. A sterile filter paper (6 mm) discs were left in the suspension and shaken well (Mie *et al*., 2014).

2.2.6.1.2 Disc Diffusion Method

In nutrient agar, pure bacteria colonies were grown at 37C˚ 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, and then PtNPs discs were placed. After that, the discs were gently pressed 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 at37C˚. A control gentamicin disc (10 μg) was used to compare with PtNPs discs (Nishanthi *et al*., 2019).

2.2.6.2 Antitumor Study of the Synthesized PtNPs

Two cell lines were obtained from the IRAQ Biotech Cell Bank Unit in Baghdad and cultivated: A-673 and MEF cells. A-673 cells are human bone cancer cells isolated from a 15-year-old woman, and MEF cells are normal mouse embryonic fibroblast cells.

2.2.6.2.1 Cell culture

The method (Freshney, 2021) was used to grow cancerous cells line as follows:

1- Cells of each line were placed in a culture container with a diameter of 25 cm^2 containing RBMI-1640 culture medium and 10% calf B serum.
2- The containers containing the cell suspension and culture medium were incubated in a 5% CO2 incubator at 37C˚ for 24 hours.
3- After a day of incubation, and when it was confirmed that there was growth in the cell culture and that it was free of contamination, secondary cultures were conducted.
4- The cells were examined using an inverted microscope to ensure their viability and growth to approximately 500 to 800 thousand cells/ml.
5- The cells were transferred to the growth booth, and the culture medium was disposed of.
6- The cells were washed with PBS solution and then discarded, and the process was repeated twice for 10 minutes each time.
7- A sufficient amount of trypsin/version enzyme was added to the cells, incubated for 30-60 seconds at 37°C, and monitored until they changed from a monolayer of cells to single cells. The enzyme was stopped by adding a new culture medium containing serum.
8- The cells were collected in centrifugal tubes and placed in a centrifuge at 2000 rpm/min for 10 minutes at room temperature to precipitate the cells and eliminate the trypsin and the used culture medium.

9- The filtrate was discarded, and the cells were suspended in a fresh culture medium containing 10% serum.

10- Examine the number of cells by taking a specific volume of the cell suspension and adding to it the same volume of Trypan Blue stain to determine the number of cells and their vitality by using a Hemacytometer slide, according to the equation:

$$C = N \times 10^4 \times F \backslash ml$$

Since:-

C = number of cells in one ml of solution

N = number of cells in the slide

F = dilution factor

10^4 = Slide Dimensions

11- The percentage of cell vitality in the sample was also calculated by using a Hemacytometer slide according to the equation:

Live cell viability = (live cells) \ (dead cells) x 100

12- The cell suspension was distributed in new containers and then incubated in a 5% CO2 incubator at 37C˚ for 24 hours.

2.2.6.2.2 Cytotoxic assay of biosynthesized platinum nanoparticles on cancer cell lines

The concentrations of PtNPs of 10, 20, 40, and 80 µg/ml were used and sterilized using a filter unit with a diameter of 0.22 µm under sterile conditions. All prepared concentrations were used immediately after completing the preparation process.

1- Prepare the cell suspension by treating the contents of a 25 cm^2 tissue culture container with trypsin/versine solution after emptying the old culture medium and gently moving the bottle, then incubating in the incubator at a temperature of 37 C° for 10 minutes, then 20 ml of the culture medium containing serum was added to it. The cell suspensions were mixed well, and 0.2 ml was transferred to each hole of the flat-bottomed plate for tissue culture using an automatic fine pipette.
2- The plate was left in the incubator at a temperature of 37 C° for 24 hours until the cells adhered to the hole, after which the old culture medium was disposed of in the holes, and 0.2 ml of the previously prepared concentrations of the extract was added with three replicates for each concentration, in addition to that three replicates were made for control (cell suspension only) plates were incubated at 37C°.
3- After 24 hours of exposure time, remove the plate from the incubator and add crystal violet stain solution to all holes containing the cells at a rate of 100 µl for each hole.
4- The plate was returned to the incubator for 20 minutes, after which it was taken out, its contents were removed, and the cells were washed with water until the excess stain was removed, as the living cells take the stain while the dead ones do not.
5- The results were read using the ELISA with a wavelength of 492 nm.

The inhibiting ratio was calculated according to the equation:

Percentage of cell inhibition = (absorbance reading of control cells - absorbance reading of treated cells for each concentration/absorbance reading of control cells) x 100

2.2.7 Statistical analysis

The results were statistically analyzed using the Graph pad Prism Version 6 analysis system and ANOVA test. The means were compared with the Duncan Multiplex experiment with significant differences at a probability level of $P \leq 0.05$.

Chapter Three

Results & Discussions

3. Results and Discussions

3.1 Isolation of bacteria from soil samples

3.1.1 Culturing samples and morphological characteristics

In the present study, the samples were collected from soil sources, in which thirty soil samples were gathered from different regions. Cultured on SCN agar media and purified on YEGA (selective media). Eleven colonies were selected, and the results showed six isolates of Actinomycetes bacteria from the eleven bacteria isolates from the soil. They were: *Streptomyces omiyaensis ,Streptomyces macrosporeus ,Streptomyces thermolilacinus , Streptomyces fradiae , Streptomyces zaomyceticus , Streptomyces ziwulingensis.* Actinomycetes isolates are characterized depending on their differences in the colony, morphology, and other features like variations in colors (black, white, pink, gray, purple, and yellow), the variety in their texture from powdery to gelatinous, and their ability to produce and form the mycelia structures. The differences in color of the aerial mycelia of the isolates and those of the pigments they produce may indicate the diversity of Actinomycetes isolates in the sites investigated.

3.1.2 Microscopic examination

The light microscope was used to distinguish between Actinomycetes and other bacteria. Firstly, by using a Gram stain to differentiate between Gve+ and Gve- bacteria, and secondly, by using a cotton blue stain, we get Actinomycetes isolates. They were observed under a microscope for their mycelial structure and hyphae arrangements on the mycelia. The spore-bearing hyphae of the strains were straight to flexible, as shown in Figure (3.1). The number of spores varied between strains, which indicated the diversity in the long chains of spores.

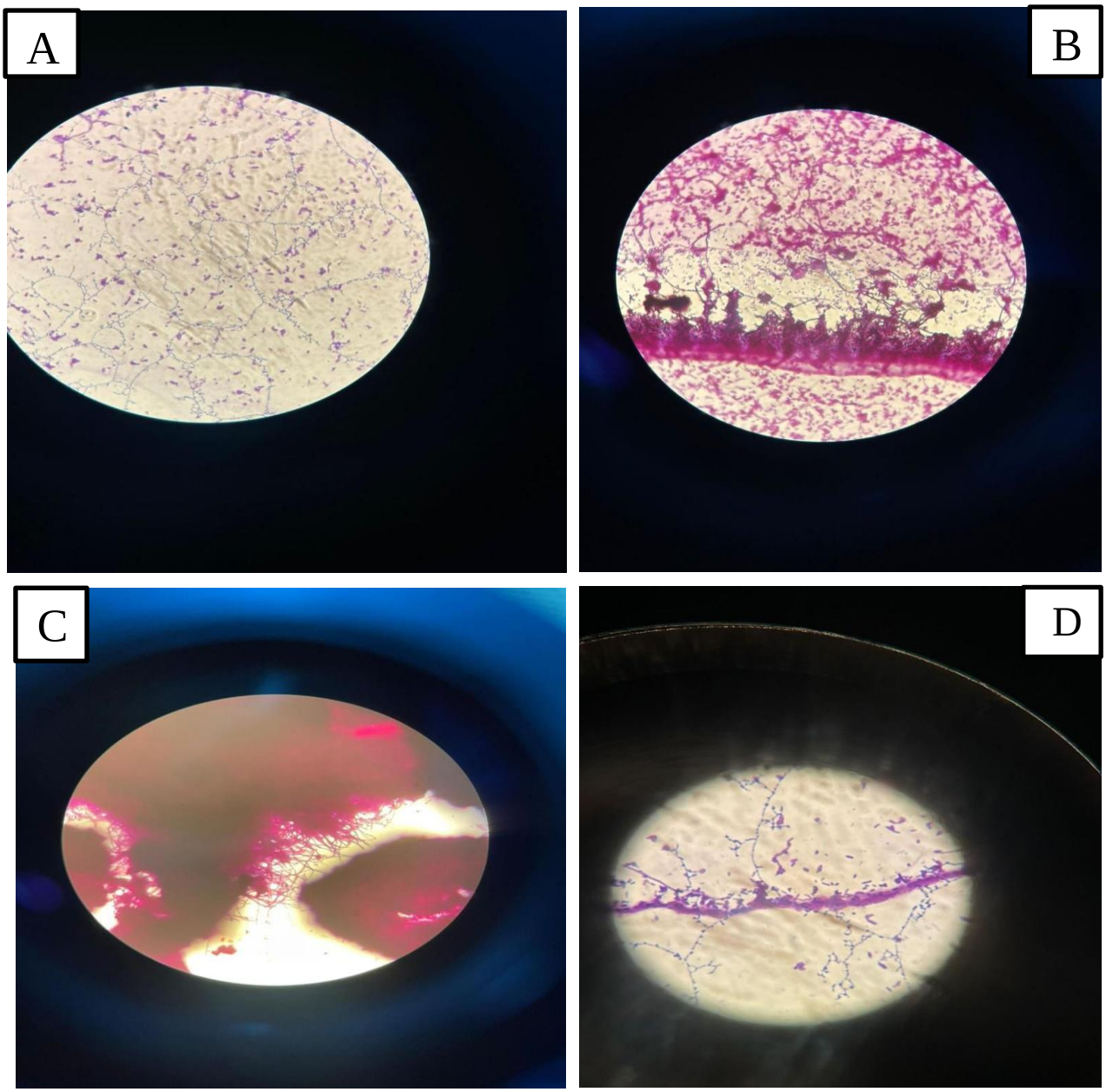


Figure (3.1): A, B, C, and D represent Actinomycetes isolates in the present study staining by Gram stain under light microscope magnification 40 x.

Many studies have indicated the isolation of Actinomycetes from soil samples (Sapcota *et al*., 2020); this study was carried out to find the antimicrobial-producing Actinomycetes from several geographical regions, the isolated and identified strains were: genus *Streptomyces* sp. (70.7%), *Nocardia* sp. (19.5%), and *Micromonospora* sp. (9.5%). This study showed that all the isolates were slow-growing, aerobic, with different colored aerial and substrate mycelia; most of them also produced pigments. Budhathoki and Shrestha (2020) have registered the isolation of Actinomycetes from different regions of soil; the samples were collected in this study from different environmental locations, which include forest land, river-bank land, agricultural lands, and damp lands; this study improved that Actinomycetes have broad-spectrum antibacterial activity and especially inhibitory activity against drug-resistant strains

3.2 The molecular identification of isolated bacteria

3.2.1 The extraction of bacterial genomic DNA

The amplification of genomic DNA by PCR was followed by agarose gel electrophoresis, and the results confirmed the presence of extracted DNA from isolates obtained from soil samples.

3.3 PCR amplification of 16S rDNA gene

The identities of bacterial isolates were determined using the polymerase chain reaction (PCR) technique to amplify the 16S rDNA gene (1500 bp). The BLAST program was used to analyze the DNA sequencing results of the bacterial isolates and match them with their reference strains in the Gen Bank. The Gen Bank database preserves and stores the genomic DNA's nucleotide sequences (Abd-Elnaby *et al*., 2016). The current study identified (11) bacterial strains, six of which belonged to *Streptomyces* sp., as shown in Figure (3.2).

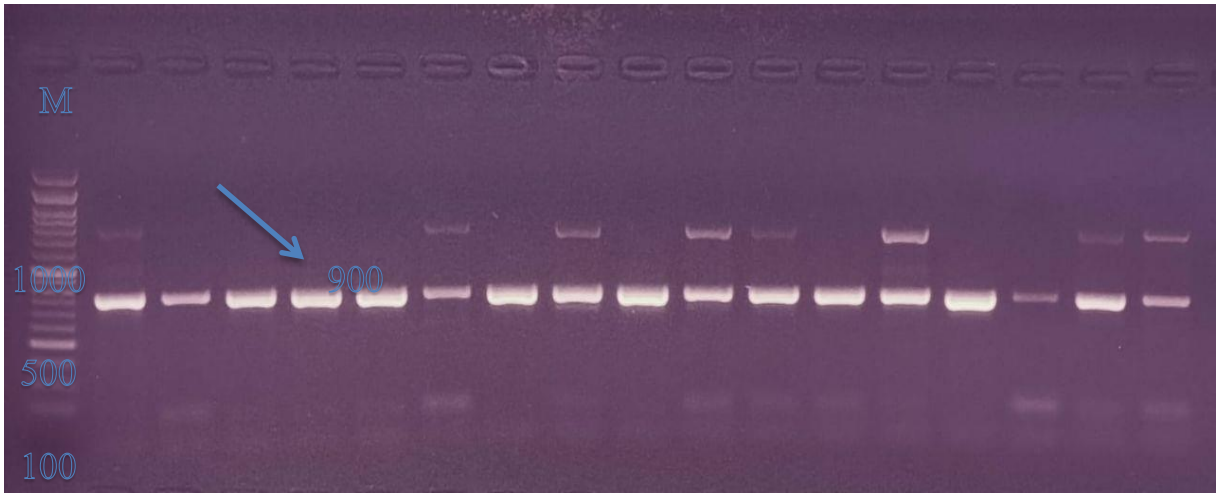


Figure (3.2): The gel electrophoresis results of PCR products, the 16S rDNA gene electrophoresis exhibit amplified genomic DNA extracted from bacterial species obtained from soil samples. Electrophoresis on 1% Agarose Gel at 60 volts for 45min. Photograph Using UVP system, M:100bp DNA ladder.

The vital feature of the 16S rDNA gene is its stability, which does not change with time and is found in almost all bacterial species. Its length (about 1500 bp) is large enough, which makes it useful for informatics objectives (Palma-Jiménez *et al.*, 2018). Many studies have shown that it is broadly used to identify and taxonomy bacteria.

In the current study, the sequencing results of the PCR product of the targeted sample were edited, aligned, and analyzed. The present study included six isolates and the respective sequences in the reference database within the targeted locus. These isolates were screened to amplify the 16S rRNA sequences of Streptomyces sp partially partially. The sequencing reactions indicated the exact identity after performing NCBI blastn for these PCR amplicons. Concerning the ribosomal amplified fragments, the NCBI BLASTn engine showed an entire sequence similarity of 100% between the five samples (assigned Z1, Z2, Z3, Z4, and Z12) and the nucleic acid sequences of *Streptomyces sp*. One sample (assigned Z8) showed 99% similarity with the same target. The accurate positions and other details of the retrieved PCR

fragments were identified, the total length of the targeted locus was localized in the NCBI server, and the positions of the targeted locus were also confirmed within the most homologous bacterial target.

When the 16S rDNA gene sequences of bacterial isolates were compared with sequences in Gen Bank, the NCBI BLASTn search engine showed that isolates Z1, Z2, Z3, Z4, Z8 and Z12 were similar to *Streptomyces omiyaensis* strain HVK1, *Streptomyces macrosporeus*, *Streptomyces thermolilacinus strain NIOT_MBCT17B*, *Streptomyces fradiae*, *Streptomyces zaomyceticus*, *Streptomyces ziwulingensis* respectively as shown in Table (3.1).

Table(3.1): Actinomycetes isolates in the soil samples identified using the 16S rDNA gene.

Isolate code	Closet bacteria	Accession no. of closet bacteria	Similarity with reference strains
Z1	*Streptomyces omiyaensis*	LC779669.1	100%
Z2	*Streptomyces macrospores*	X808271.1	100%
Z3	*Streptomyces thermolilacinus*	MN181426.1	100%
Z4	*Streptomyces fradiae*	MN901087.1	100%
Z8	*Streptomyces zaomyceticus*	MN826242.1	99%
Z12	*Streptomyces ziwulingensis*	OQ067007.1	100%

Interestingly, the alignment results of five amplified samples revealed the presence of no nucleic acid variations in the analyzed sample compared with the most similar referring reference nucleic acid sequences. In contrast, the Z8 sample showed three nucleic acid variations (77G>A, 86C>T, 156T>C, and 276T>C) with the same target. These isolates with 99% similarity were recorded as new strains in the Gen Bank database. The names and accession numbers of these strains are listed in Table

(3.1). Z8 isolates were deposited in NCBI with unique GenBank accession numbers obtained for this sample to represent PP761597.

Description	Query Cover	E value	Per. Ident	Acces
Streptomyces sp. strain KK 16S ribosomal RNA gene, partial sequence	94%	8e-143	98.97%	OR539445.1
Streptomyces roseicoloratus strain T14 chromosome, complete genome	94%	8e-143	99.31%	CP133762.1
Streptomyces narbonensis strain F31 16S ribosomal RNA gene, partial sequence	90%	8e-143	98.97%	KU324447.1
Streptomyces phaeochromogenes strain F19 16S ribosomal RNA gene, partial sequence	90%	8e-143	98.97%	KU324440.1
Streptomyces gardneri strain ChNPU F3 16S ribosomal RNA gene, partial sequence	86%	8e-143	98.97%	KX349221.1
Streptomyces narbonensis strain T5 16S ribosomal RNA gene, partial sequence	90%	8e-143	98.97%	KU317901.1
Streptomyces venezuelae gene for 16S ribosomal RNA, partial sequence, strain: DKCM0301	94%	8e-143	98.97%	LC133071.1
Streptomyces sp. R311 16S ribosomal RNA gene, partial sequence	88%	8e-143	98.97%	KX010107.1
Streptomyces sp. SUK25 16S ribosomal RNA gene, partial sequence	87%	8e-143	98.97%	KU555932.1
Streptomyces zaomyceticus strain SR27 16S ribosomal RNA gene, partial sequence	91%	8e-143	98.97%	OQ918325.1
Streptomyces sp. 3HB-1o 16S ribosomal RNA gene, partial sequence	85%	8e-143	98.97%	KU052949.1
Streptomyces sp. M2104 16S ribosomal RNA gene, partial sequence	90%	8e-143	98.97%	KT970737.1
Streptomyces sp. JSM 147805 16S ribosomal RNA gene, partial sequence	88%	8e-143	98.97%	KR817779.1
Streptomyces sp. JSM 147762 16S ribosomal RNA gene, partial sequence	89%	8e-143	98.97%	KR817772.1
Streptomyces bikiniensis strain RP15 16S ribosomal RNA gene, partial sequence	90%	8e-143	98.97%	KR676539.1
Streptomyces purpureus strain OILPAINTS 9 16S ribosomal RNA gene, partial sequence	96%	8e-143	98.97%	OQ726573.1
Streptomyces mauvecolor strain BCCO 10_769 16S ribosomal RNA gene, partial sequence	88%	8e-143	98.97%	KP718571.1
Streptomyces mauvecolor strain BCCO 10_317 16S ribosomal RNA gene, partial sequence	88%	8e-143	98.97%	KP718570.1
Streptomyces sp. FSRo5 16S ribosomal RNA gene, partial sequence	90%	8e-143	98.97%	KP900837.1
Streptomyces bikiniensis gene for 16S ribosomal RNA, partial sequence, strain: 13667e	88%	8e-143	98.97%	LC072711.1
Streptomyces michiganensis strain IHB B 10204 16S ribosomal RNA gene, partial sequence	91%	8e-143	98.97%	KR233777.1

Figure(3.3): Sequences producing significant alignments for *Streptomyces zaomyceticus* (Z8) using Blast site.

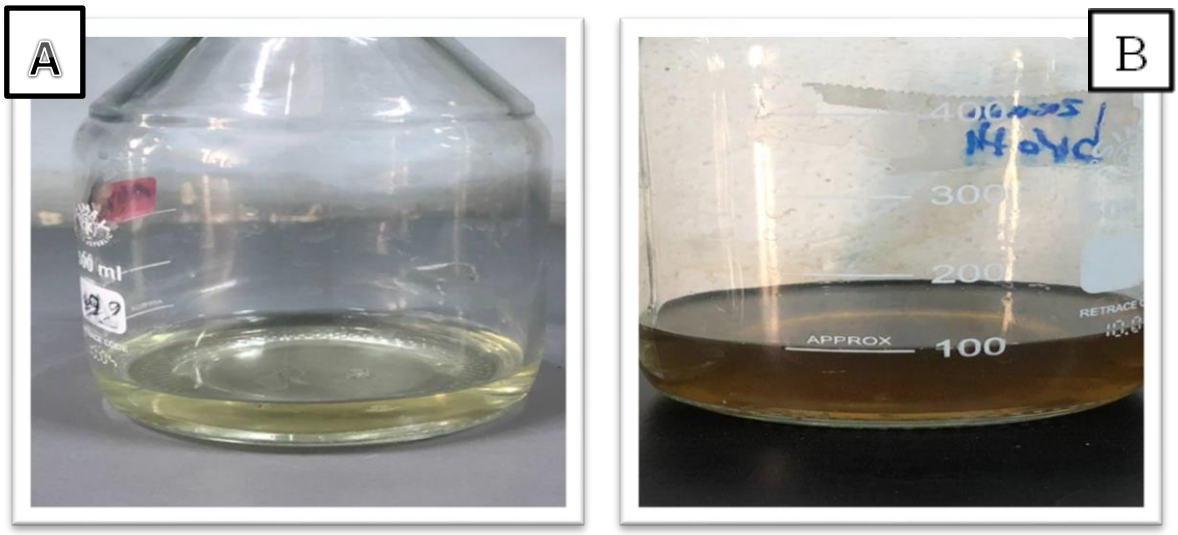
Molecular identification of soil bacteria demonstrated that the bacterial populations in the present study varied considerably; for instance those obtained by (Shrestha *et al*., 2021) they were isolated different isolates from soil samples in Nepal, 12 isolates were found to be *Micromonospora* sp., 7 were *Streptomyces* sp. and 9 were *Nocardia* sp. they finding that geographical diversity of Nepal is very suitable for adaptation of different species of Actinomycetes and tested the isolates as producers of antimicrobial agents. In another study by (Sarika *et al.*, 2021), they isolated Actinomycetes from coal amine in India; the results of biochemical and microscopic studies of isolates indicate the potential isolate strains belong to *Streptomyces* genus; in this study, they tested the supernatant of all the isolates for antimicrobial and antifungal activities. The current study showed that the

Streptomyces sp. were more abundant than other genera of Actinomycetes. Generally, all the studies showed bacterial diversity; for example, the results from the mentioned studies differed from the bacterial community in our research. The main reason microbial communities are identified by soil geology is that they are affected by different conditions, e.g., temperature, pH, and salinity, that influence the structure and metabolic activity of the microbial populations.

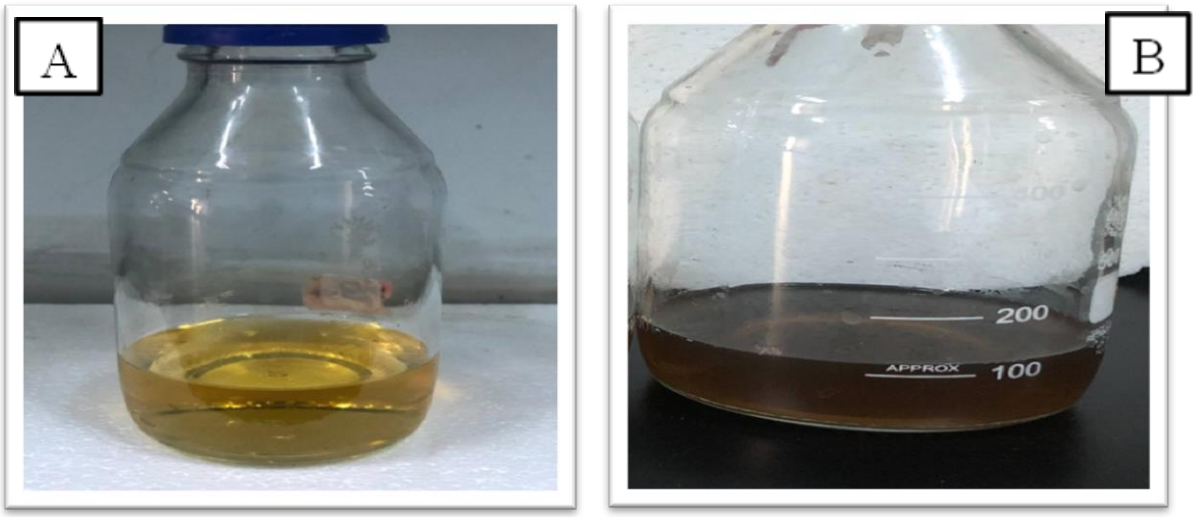
3.4 The bacterial screening for the ability to synthesize PtNPs

Only five identified species out of 6 isolates, including *Streptomyces* sp., were used to screen PtNPs production. After growing the isolated bacteria from soil samples on the fermentation media (MGYP and nutrient broth) and by obtaining bacterial filtrate (free cell supernatant), which was mixed with chloroplatinic acid hydrate dissolved in deionized water, the results showed the presence of color change after an incubation period of only two strains of bacterial isolates, which were *Streptomyces fradiae* and *Streptomyces ziwulingensis*. These results confirm a reaction between the active secondary compounds in the bacterial extract and the chloroplatinic acid hydrate. In addition, these two species can bio-sorb metal ions on their surface and reduce them to the corresponding nanoparticles through different mechanisms by enzymatic activity, including reductases, cytochromes, and metallothioneins. (Bloch *et al.*, 2021).

The reaction mixture showed a dark/greyish brown color upon reaction, as shown in Fig(3.4) and Fig (3.5), indicating the formation of PtNPs. Chloroplatinic acid hydrate was added to the cell-free supernatant (CFS), and the color changed from pale yellow to greyish brown, observed after (7-10) days of reaction, confirming the synthesis of PtNPs. The color changes that occurred are due to Plasmon Surface Resonance. These results agreed with the study of Borse *et al*. (2015), which explains the color change Because of surface plasmon resonance that showed a greyish-brown color in the solution



Figure(3.4): The color change of the culture supernatant of the bacterial strain *Streptomyces fradiae* (K). A: the supernatant of bacteria without Pt salt B: the supernatant with Pt salt after incubation for seven days.



Figure(3.5): The color change of the culture supernatant of the bacterial strain *Streptomyces ziwulingensis* (H). A: the supernatant of bacteria without Pt salt B: the supernatant with Pt salt after incubation for seven days.

Many studies have explained the production mechanism of PtNPs; Capness *et al*. (2015) proposed the participation of cytochromes and hydrogenase enzymes in the reduction process in anaerobic sulfate-reducing bacteria *Desulfovibrio alaskensis*. Attard *et al*. (2012) suggested that *E. coli* possesses various hydrogenase enzymes that catalyze the hydrogen cleavage into protons and electrons that facilitate the biosynthesis of Pt(0)NPs. In addition, Riddin *et al*. (2009) proposed the involvement

of two diverse hydrogenase enzymes in the biogenic synthesis of Pt(0)NPs in sulfate-reducing bacteria, where Pt(IV) is firstly reduced to Pt(II) using a cytoplasmic hydrogenase enzyme, followed secondly the Pt(II) reduction to Pt(0)NPs by a periplasmic hydrogenase enzyme. While Baskaran *et al*. (2017) suggested the extracellular production of Pt(0)NPs by *Streptomyces* sp. was attributed to the chloride reductase enzyme, these enzymes are involved in the nitrogen cycle responsible for reducing chloride to chlorine. Additionally, it is known that nicotinamide adenine dinucleotide-dependent chloride reductase enzymes are significant factors in the biosynthesis of NPs. The electron shuttle enzymatic metal reduction process represents the possible mechanism that might involve the reduction of Pt ions; this is agreed with the present study.

3.5 The Characterization of the Biosynthesized PtNPs

3.5.1 UV-Vis. Spectroscopy

UV-Vis spectroscopy was used immediately after the reduction of platinum; the ultraviolet-visible spectrometric measurements were performed in the range of (200-800) nm to confirm the formation and stability of PtNPs (Castro *et al*., 2015). The reduction was from Pt^{+4} to Pt^{0}, and the color of the solution was observed to change from yellow to greenish brown, which indicates that distinct platinum particles were formed as a result of the reduction of H_2PtCl_6 (Prabhu and Gajendran, 2017). In the present study, the PtNPs biosynthesized using *Streptomyces fradiae ,* showed a broad peak of this nanoparticle (362) nm wavelength. While PtNPs biosynthesized by *Streptomyces ziwulingensis* showed wavelength (378) nm, represented in (figure 3.6) and (figure 3.7)

The intense color of the distributed PtNPs was due to the absorption of the surface plasmon resonance (SPR) spectrum of the PtNPs. For this reason, the bio nanoparticles have a distinct optical absorption spectrum in the Ultraviolet-visible

range and show a distinct optical absorption spectrum in the UV-visible range (Othman *et al.*,2019; Yang *et al.*, 2023).

Table(3.2) UV-vis. Spectroscopy of Different Bacteria Synthesized PtNPs.

ID Samples	Type of PtNPs producing bacteria	Wave Length
K	***Streptomyces fradiae***	**362**
H	***Streptomyces ziwulingensis***	**378**

Several studies have confirmed that the wavelength of PtNPs is similar to the results in the present study. Castro *et al*. (2015) recorded the UV–visible spectra results from the PtNPs; the broad band was approximately 400 nm. Also, they found that a broad shape resonance might show an aggregated structure of the metal nanoparticles in the final product. Also, Prabhu and Gajendran (2017) registered the absorption spectrum of PtNPs, which peaked at 400 nm, confirming the presence of Pt nanoparticles.

Al-Radadi (2019) showed the biosynthesis of PtNPs from dates; after the production of PtNPs and the change of color, UV-vis measurements recorded an excitation of surface plasmon (SPR) vibration. The peak of surface Plasmon resonance (SPR) for the biosynthesized PtNPs in Ajwa and Barni dates were at (321and 329 nm) depending on the shape and size of PtNPs. Also, Al-Radadi (2020) registered the absorption spectrum of PtNPs synthesized with different concentrations of Anbara fruit extract, which showed a prominent peak at the wavelength (λ_{max}) of 380 nm.

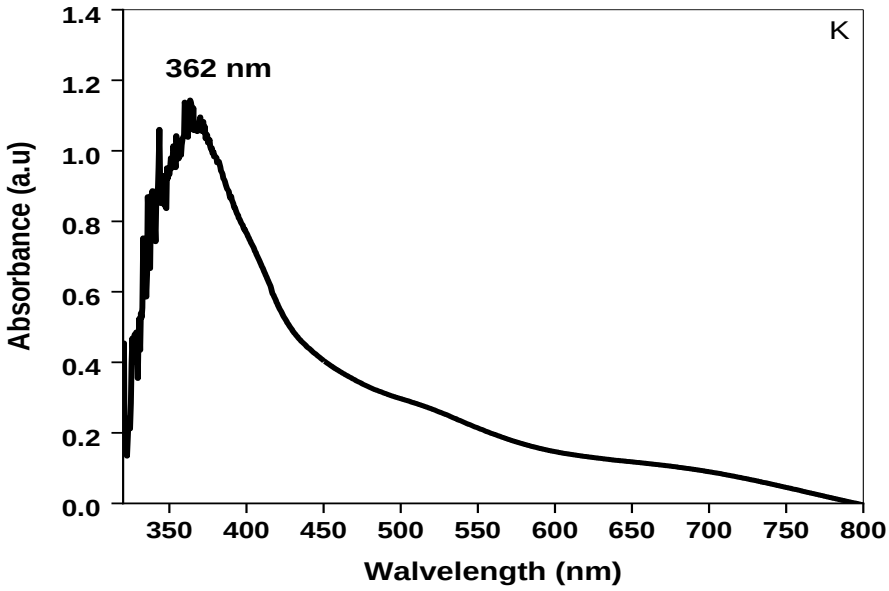


Figure (3.6): UV-visible spectroscopy of PtNPs synthesized by *Streptomyces fradiae* showed the sharpest UV–Vis spectrum peak at λmax = 362 nm.

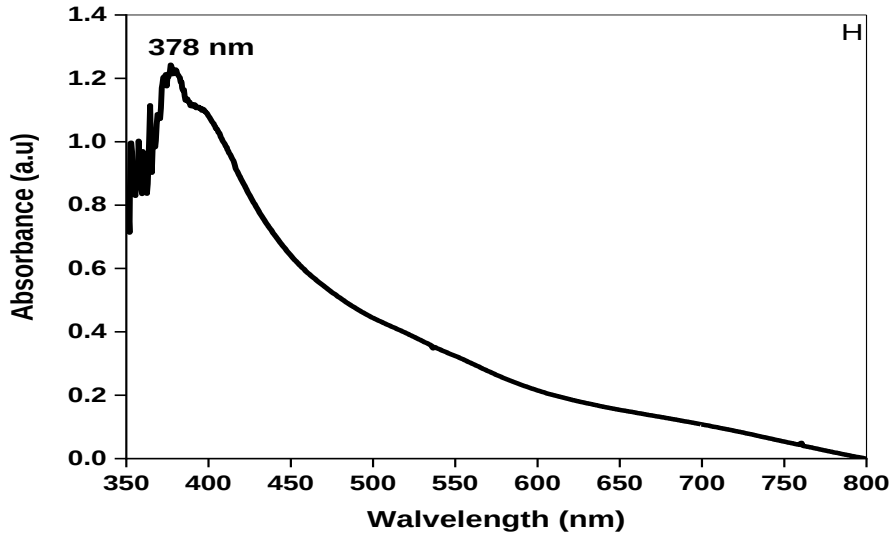


Figure (3.7): UV-visible spectroscopy of PtNPs synthesized by *Streptomyces ziwulingensis* showed the sharpest UV–Vis spectrum peak at λmax = 378 nm.

3.5.2 Zeta potential analysis

The zeta potential analysis was used to measure the charge on the PtNPs surfaces of the suspended particles in the solution and analyze the stability of NPs by the magnitude of charges by which high positive or negative values describe the higher stability of NPs. The main explanation for larger values (positive or negative) of zeta potential for these nanoparticles is that they will exert all their force to make the repulsion on each other, which will not come together or agglomerate (Manzoor *et al*., 2021). In the present study, the results were: the value of the zeta potential of the biosynthesized PtNPs by *Streptomyces fradiae* was(-28.6)mV, indicating the relative stability of PtNPs (figure 3.8). At the same time, the value of the zeta potential of PtNPs produced by *Streptomyces ziwulingensis* was(-38.1)mV (figure 3.9). These improved that the biosynthesized nanoparticles have a negative charge on their surfaces; for this reason, we can conclude that the biosynthesized PtNPs are comparatively stable and also surrounded by anionic compounds, and the zeta potential value of *Streptomyces ziwulingensis* was more negatively than the zeta value of Streptomyces *fradiae* , which is mean that the first was more stability.

Our results in the current study matched with many studies; Baskaran *et al* . (2017) demonstrated that zeta potential for the PtNPs produced by *Streptomyces* sp. is at −18.4 mV, which means that the biosynthesized PtNPs were moderately stable. This study showed that the zeta potential of the synthesized PtNPs indicates the relatively neutral nature of the NPs, and the neutral behavior of the synthesized PtNPs proposed the presence of proteins and amino acids on their surfaces. Manzoor *et al*. (2021) registered the biosynthesis of PtNPs, which were stable monodispersed nanoparticles and showed a zeta potential of −23.4 mV.

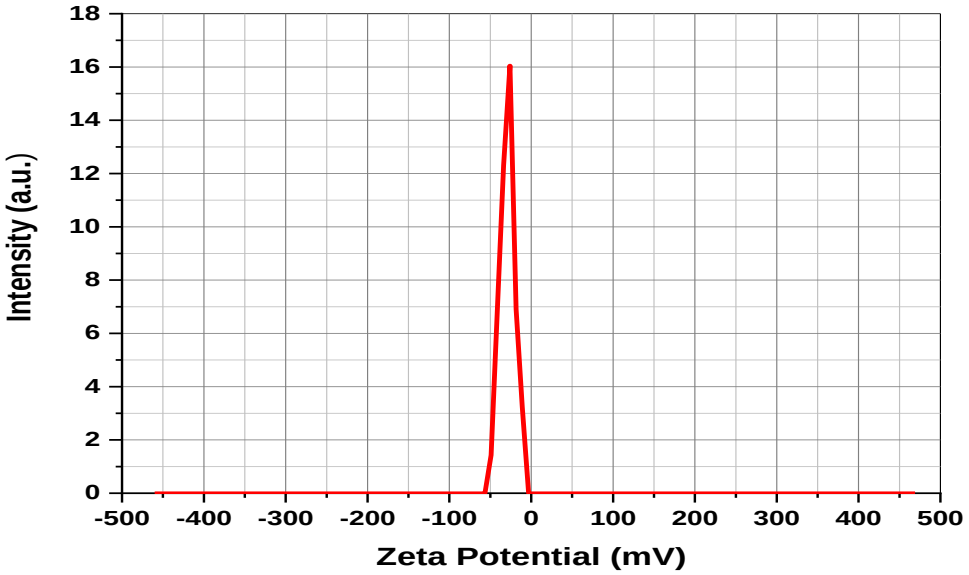
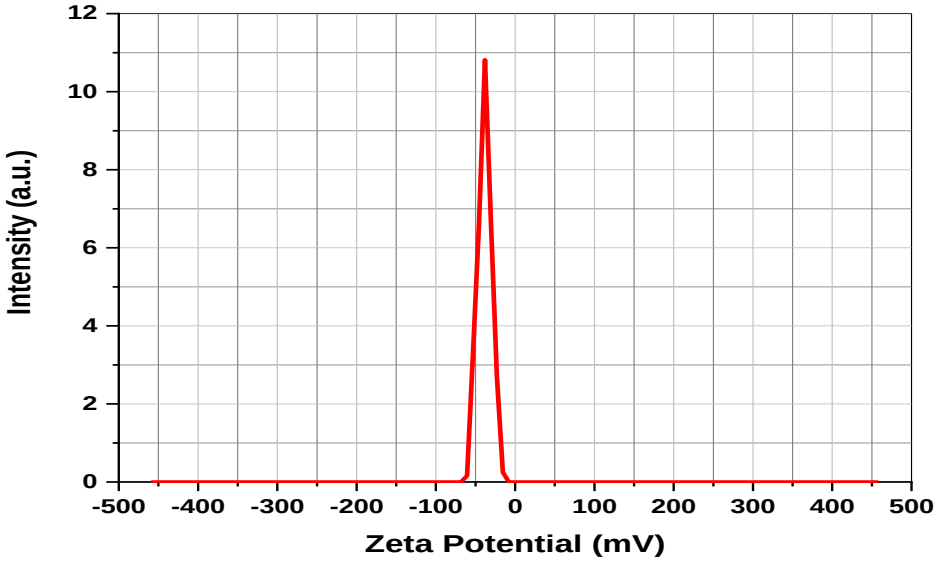


Figure (3.8): Zeta potential value of PtNPs biosynthesized using *S. fradiae* = -28.6 mV (relative stability)

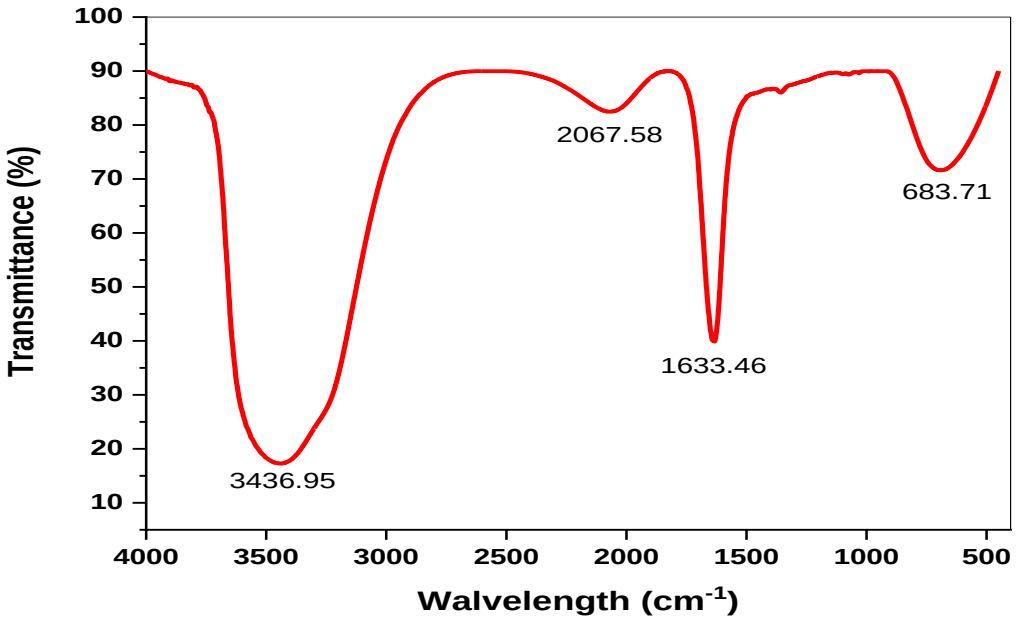


Figure(3.9): Zeta potential value of PtNPs biosynthesized using *S. ziwulingensis* = -38.1 mV (stability)

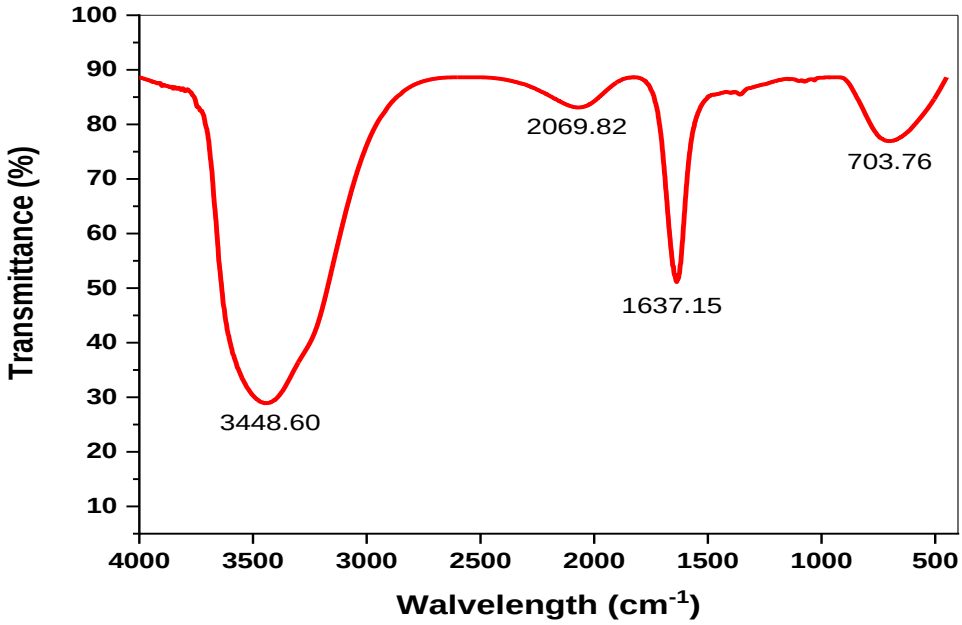
3.5.3 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra are utilized to discover the different functional groups of the biosynthesized PtNPs involved in the reduction of precursors to NPs; it can be concluded that the functional groups help in the reduction process and stabilization of the biosynthesized PtNPs. The present study showed that the FTIR spectrum of PtNPs synthesized by Streptomyces ziwulingensis has intensive vibrations to the band positions of 683, 1633, 2067, and 3436 cm^{-1}. The bands that appeared at 3436 cm^{-1} are related to (O–H) stretching (intramolecular hydrogen-bonded OH), the band that appeared at 2067 cm^{-1} may show the presence of CO_2, and the band at 1633 cm^{-1} characterizes the amine group. Also, the band at 683 cm^{-1} is due to alkyl halide stretching (Figure 3.9). In addition, the PtNPs produced by the Actinomycetes Streptomyces fradiae, the FTIR spectrum of these nanoparticles shows different bands at 3448 cm^{-1}, 2069 cm^{-1}, 1637 cm^{-1}, and 703 cm^{-1}. The band at 3448 and 1637 cm^{-1} may attributed to the (–C=C– and free N–H) vibrations that could be assigned to the heterocyclic compounds like proteins. The band of 2069 cm^{-1} refers to the presence of an amine functional group (R–N=C), while the band of 703 cm^{-1} is assigned to alkyl halides (C–X), the functional group chloride (Figure 3.10).

Many studies agreed with the current research; Dobrucka (2019) showed the biosynthesis of PtNPs, in which the FTIR spectra of samples exhibited significant bands at 3311 cm^{-1}, 2110 cm^{-1}, 1634 cm^{-1}, 607 $cm^{-1,}$ and 387 cm^{-1}. Baskaran *et al*. (2017) reported the FTIR spectrum of absorption peaks at the band positions of 676 cm^{-1}, 1637 cm^{-1}, 2081 $cm^{-1,}$ and 3434 cm^{-1}; this study corresponded with our findings.



Figure(3.10): The FTIR spectrum of biosynthesized PtNPs using *S. ziwulingensis*



Figure(3.11): The FTIR spectrum of biosynthesized PtNPs using *S. fradiae*

3.5.4 Field emission Scanning Electron Microscope(FESEM)

The surface morphology, composition, size, and shape of PtNPs synthesized from the isolated bacteria under study were examined by field emission scanning electron microscopy (FESEM). The results showed that the NPs biosynthesized by *Streptomyces* sp. were spherical, which is due to the presence of many different groups of phytochemicals that help in the reducing and stabilizing of the NPs during the formation process (Baskaran *et al*., 2017). In the current study, the PtNPs produced using *Streptomyces ziwulingensis* (H) were spherical with size in (21-65)nm, as shown in figure(3.12). The particle size distribution was 9.76 nm, which confirms that most particles present between 21 and 65 nm. At the same time, the FESEM results of PtNPs biosynthesized using *Streptomyces fradiae* (k) confirm that the particles were spherical with a size between (17-42) nm, and the size distribution was 5.55 nm (figure 3.13). These findings agreed with many studies; Dobrucka (2016) showed the size of biosynthesized PtNPs was between(20–30) nm, and the NPs were agglomerated, which could perform a better evaluation of the shape, size, and morphologies of the nanoparticles.

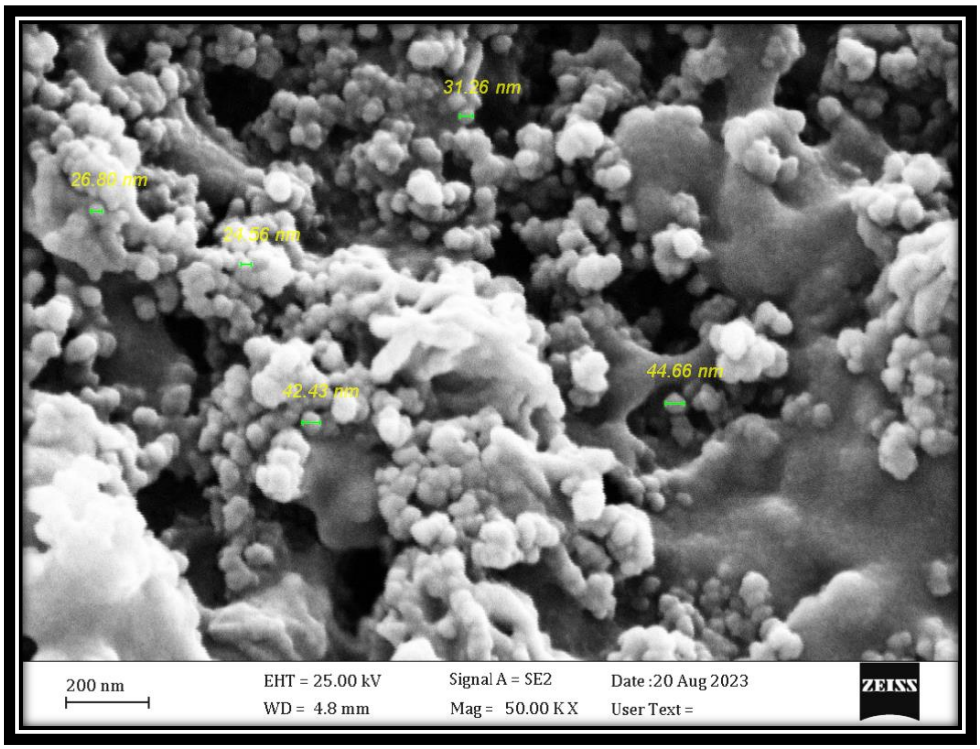
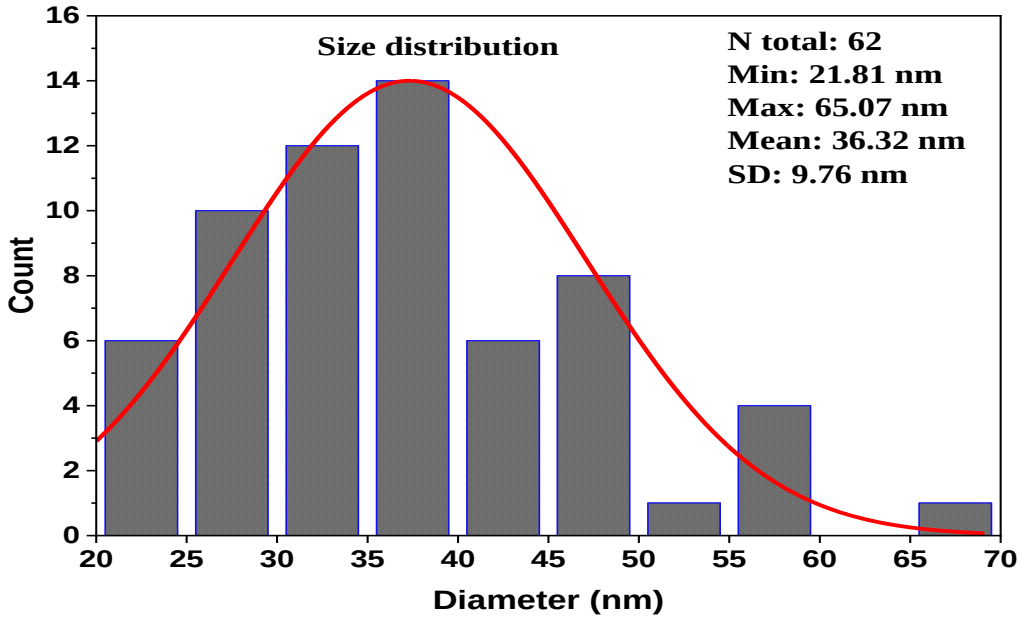


Figure (3.12) : The FESEM image of PtNPs synthesized using *S. ziwulingensis* (H).



Figure(3.13): The histogram of the size distribution of *S. ziwulingensis*

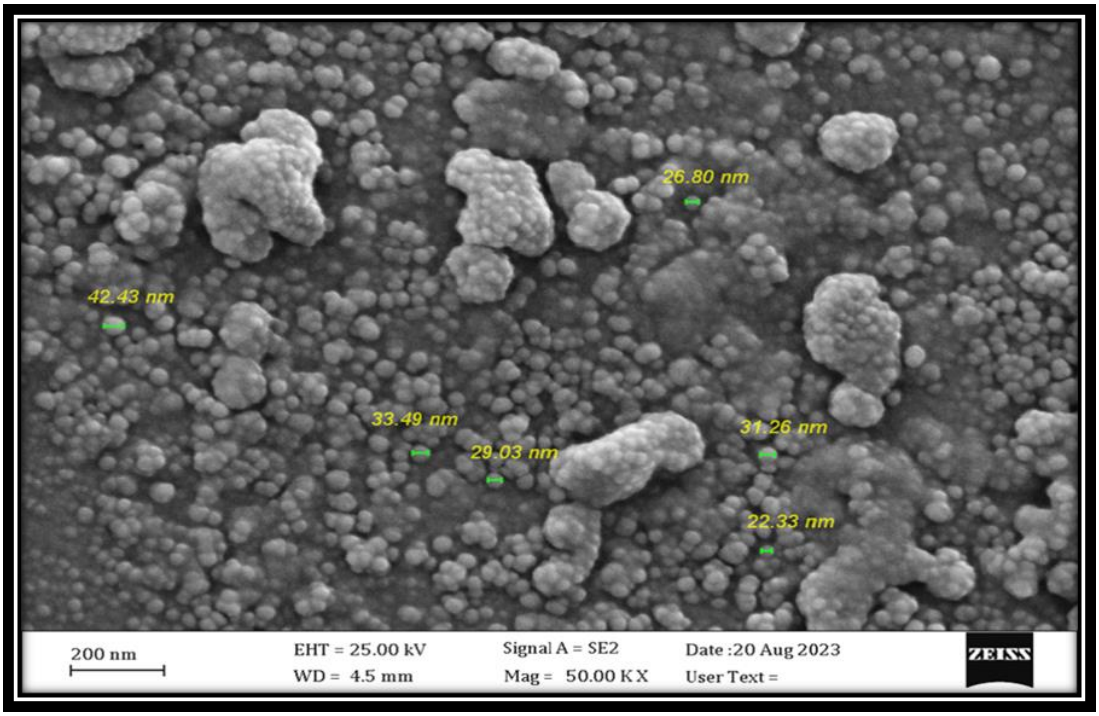
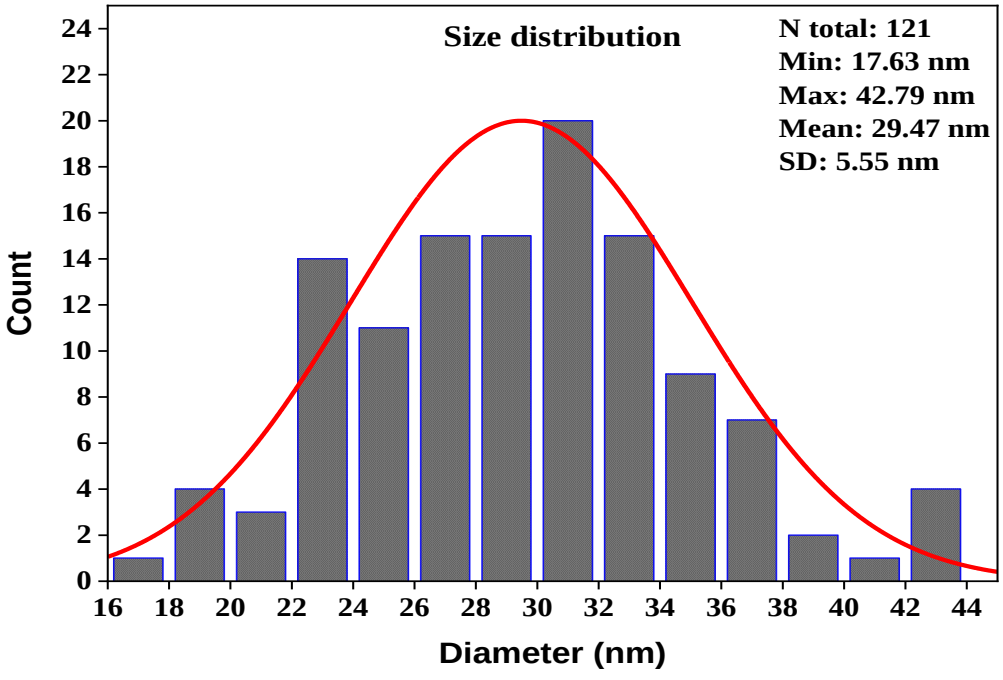


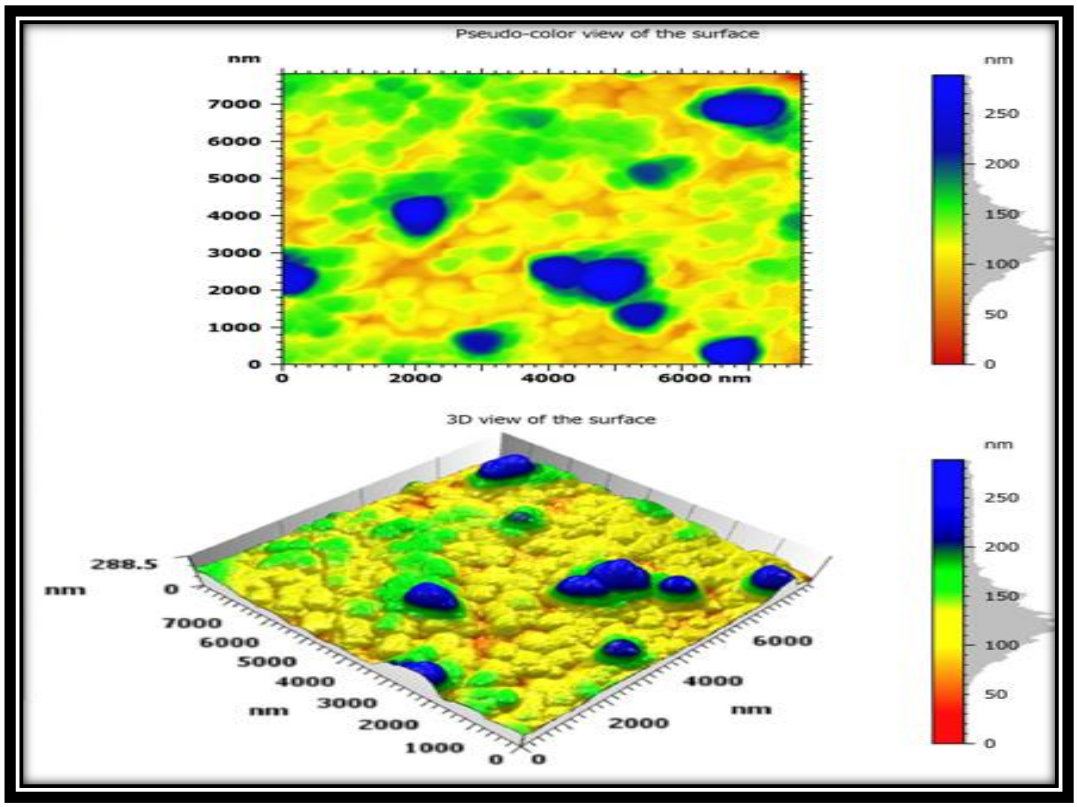
Figure (3.14): The FESEM image of PtNPs synthesized using *S. fradiae* (K).



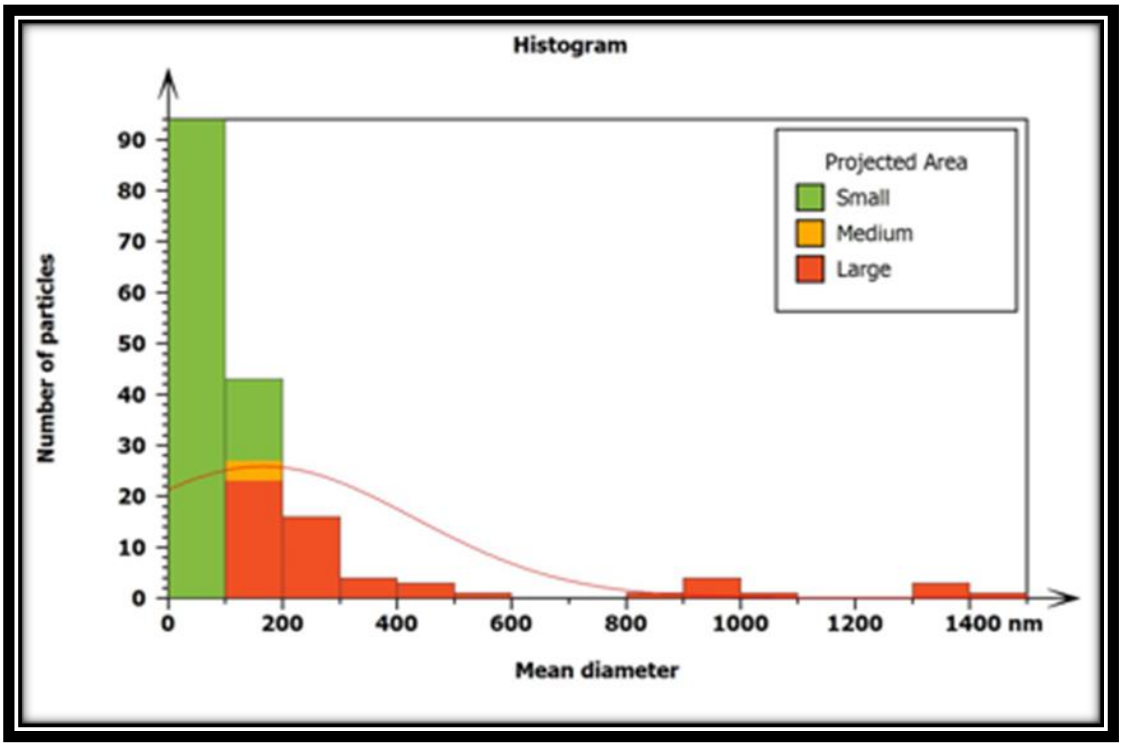
Figure(3.15): The histogram of the size distribution of *S. fradiae*

3.5.5. Atomic force microscopy (AFM)

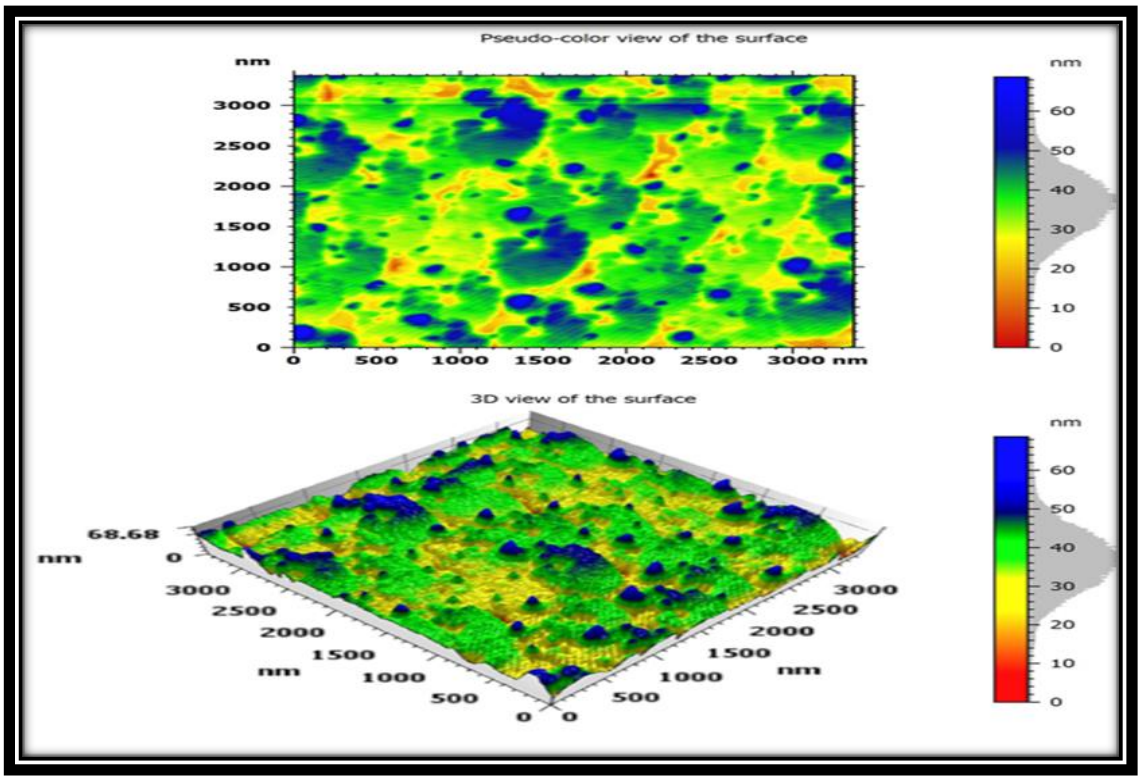
Atomic force microscopy is used to study and understand the shapes, topography, roughness, and protrusions of surfaces through this examination, in which their heights and surface structure represent the particles, which makes it possible to perform quantitative measurements to know the surface structure and access two- and three-dimensional images and analyze them from different sides **(**Salman & Abd, 2021). In the current study, the AFM results for PtNPs synthesized by *Streptomyces ziwulingensis* are shown in figure (3.16). According to the AFM findings, PtNPs had a mean diameter of about 165 nm, and the particle size was about (11.35- 1442 nm). Furthermore, the AFM's two- and three-dimensional images demonstrated the synthesized PtNPs' spherical shape and uniform distribution, which were made possible by TEM and FESEM micrographs. The AFM results for PtNPs produced using *Streptomyces fradiae* were that PtNPs had a mean diameter (41.66 nm), and the Particle size was between (4.89- 227.1 nm); the images showed a spherical shape. We could consider the material's surface rough based on atomic force microscopy (AFM) images because it contains peaks of different dimensions. The surface roughness will increase the effectiveness of killing bacteria and cancer cells (Hassan *et al*., 2023).



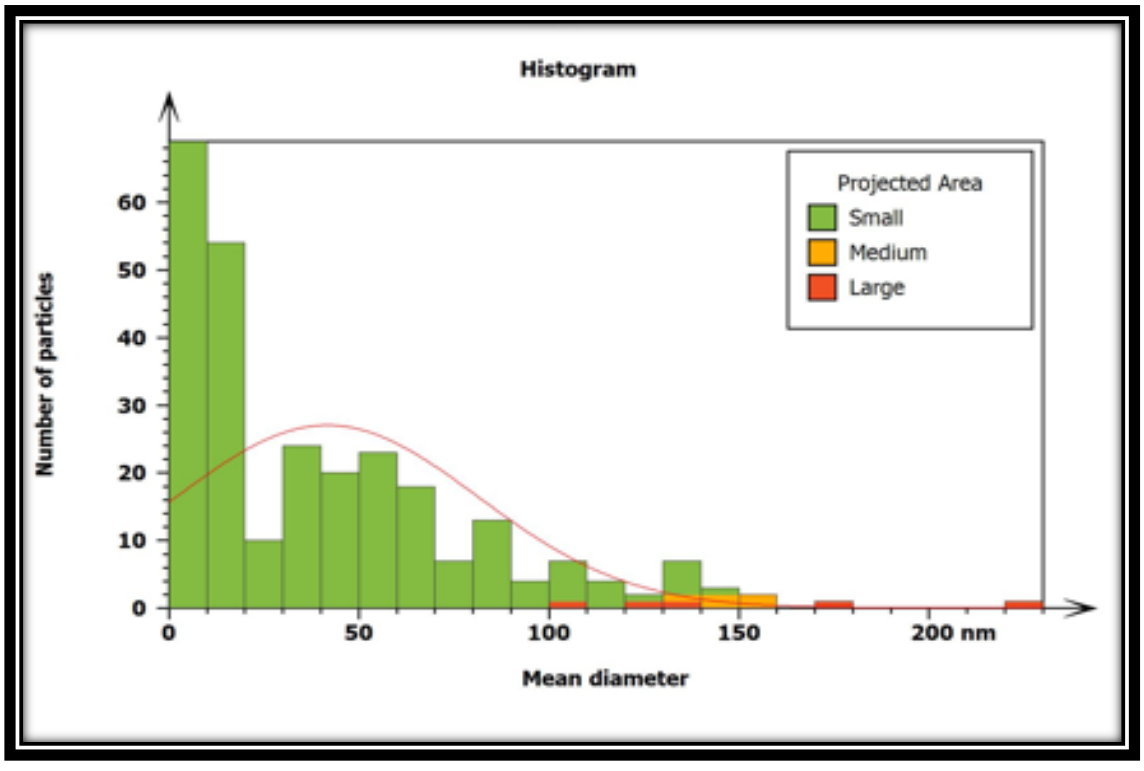
Figure(3.16): 2D and 3D images of AFM for PtNPs biosynthesized by *S. ziwulingensis* (H).



Figure(3.17): The mean diameter of AFM for PtNPs produced by *S. ziwulingensis* (H).



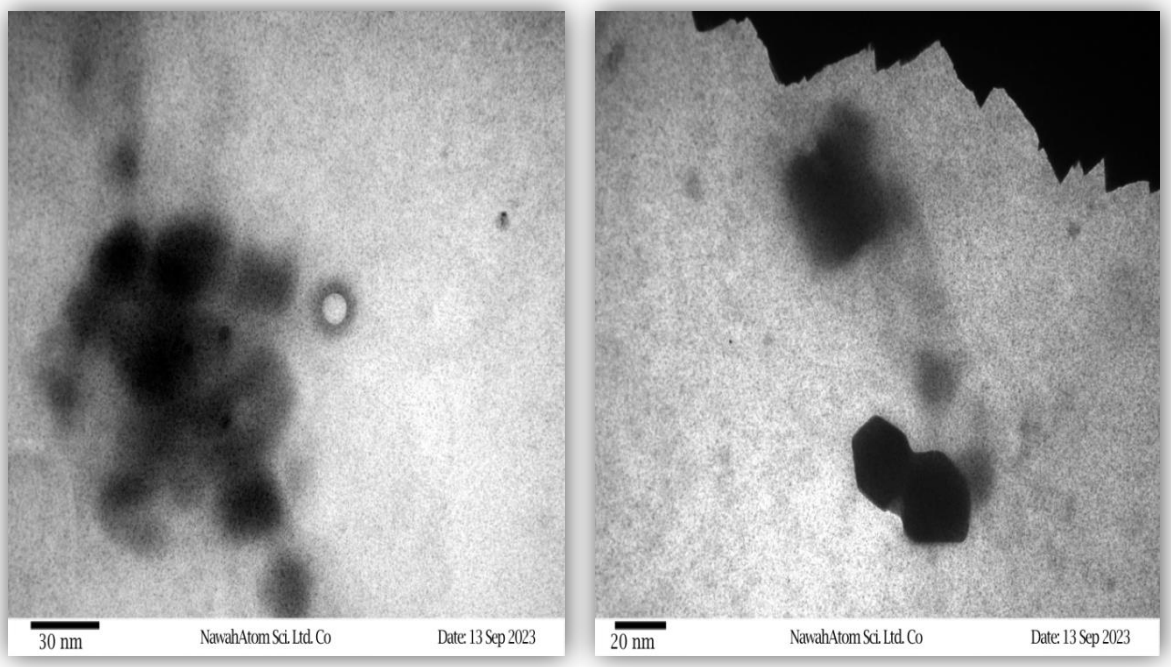
Figure(3.18): 2D and 3D images of AFM for PtNPs synthesized by *S. fradiae* (K)



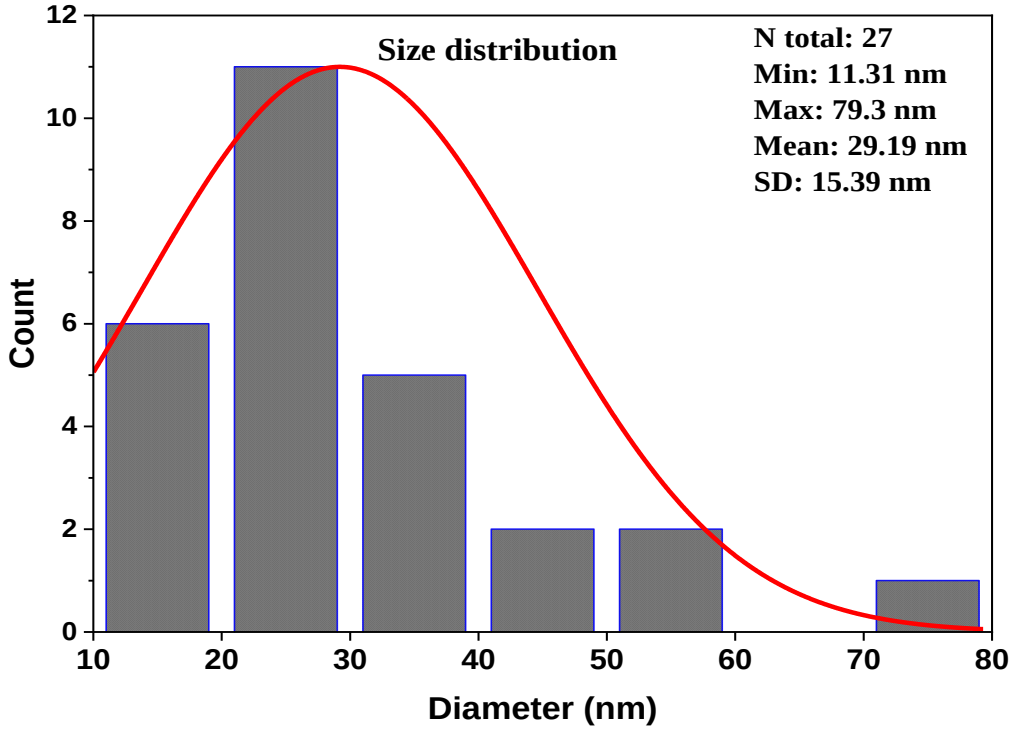
Figure(3.19): The mean diameter of AFM for PtNPs produced by *S. fradiae* (K).

3.5.6. Transmission electron microscopy (TEM)

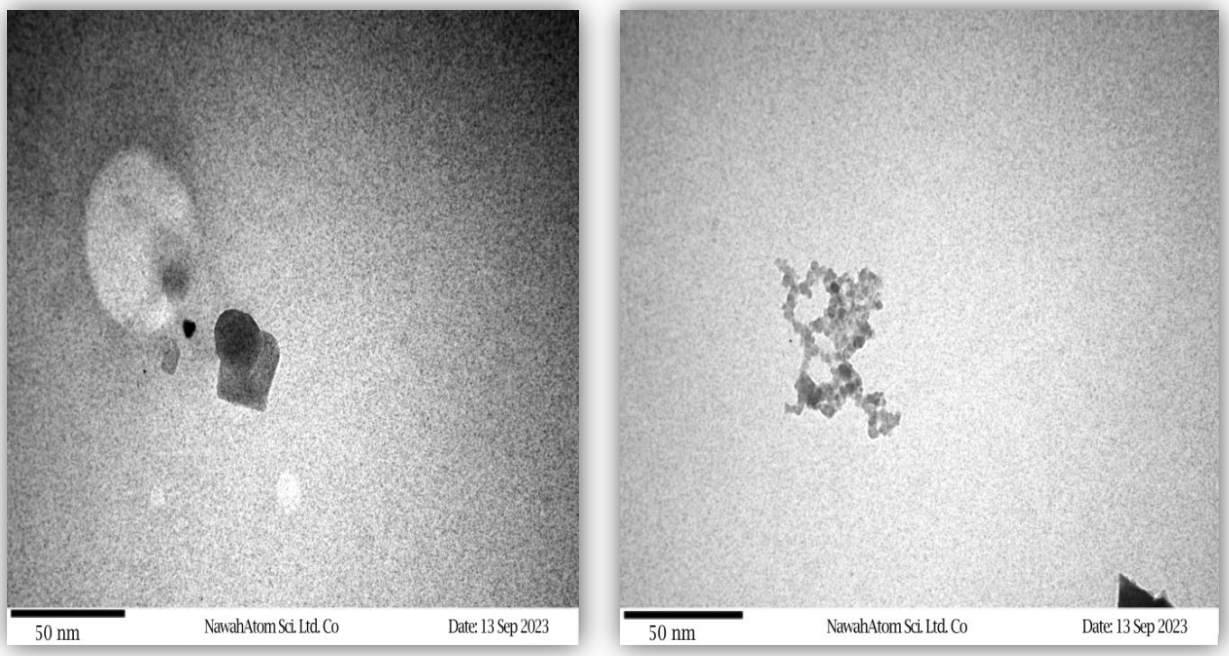
The TEM analysis observed the size and morphology of the PtNPs. The TEM image of PtNPs synthesized by *Streptomyces ziwulingensis* (H) confirmed that they were monodisperse and spherical. Also, the histogram of PtNPs size distribution showed that their sizes were between 11.31 and 79.3 nm, with mean particle sizes of 29.19 nm and size distribution of about 15.39 nm. In addition, the TEM image of PtNPs biosynthesized by *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 a somewhat uniform spherical shape and are well distributed within the 20–50 nm size range. The size ranges obtained from the TEM results also agreed with the other topographical and analytical results (Baskaran *et al*., 2017).



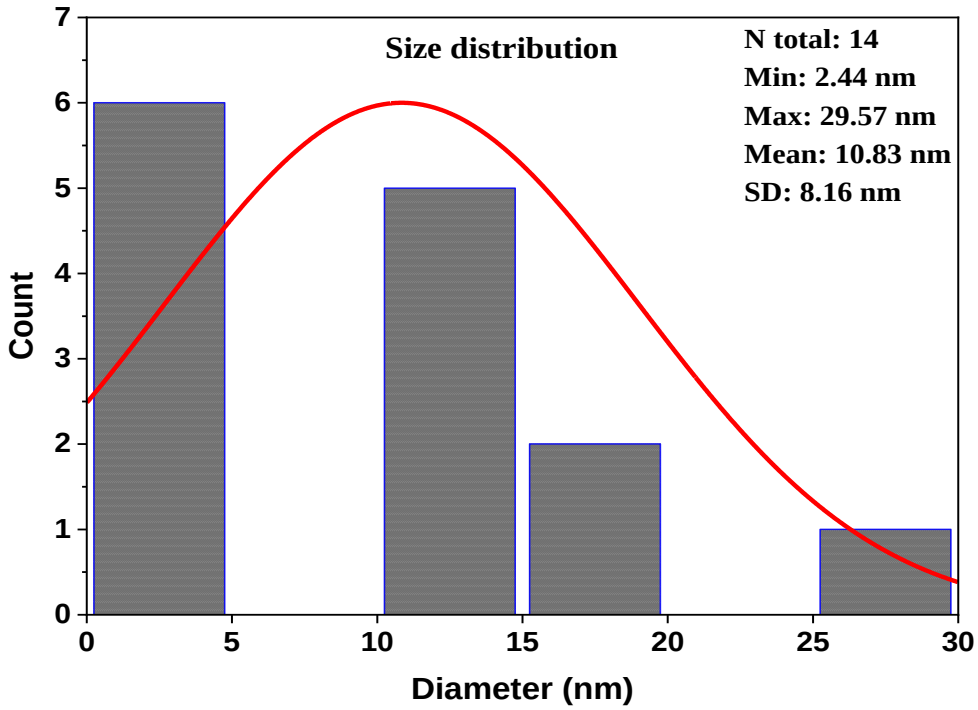
Figure(3.20): The TEM images of PtNPs synthesized using *S. ziwulingensis* (H).



Figure(3.21): The size distribution of TEM for *S. ziwulingenesis*



Figure(3.22): The TEM images of PtNPs synthesized using *S. Fradiae*(K).

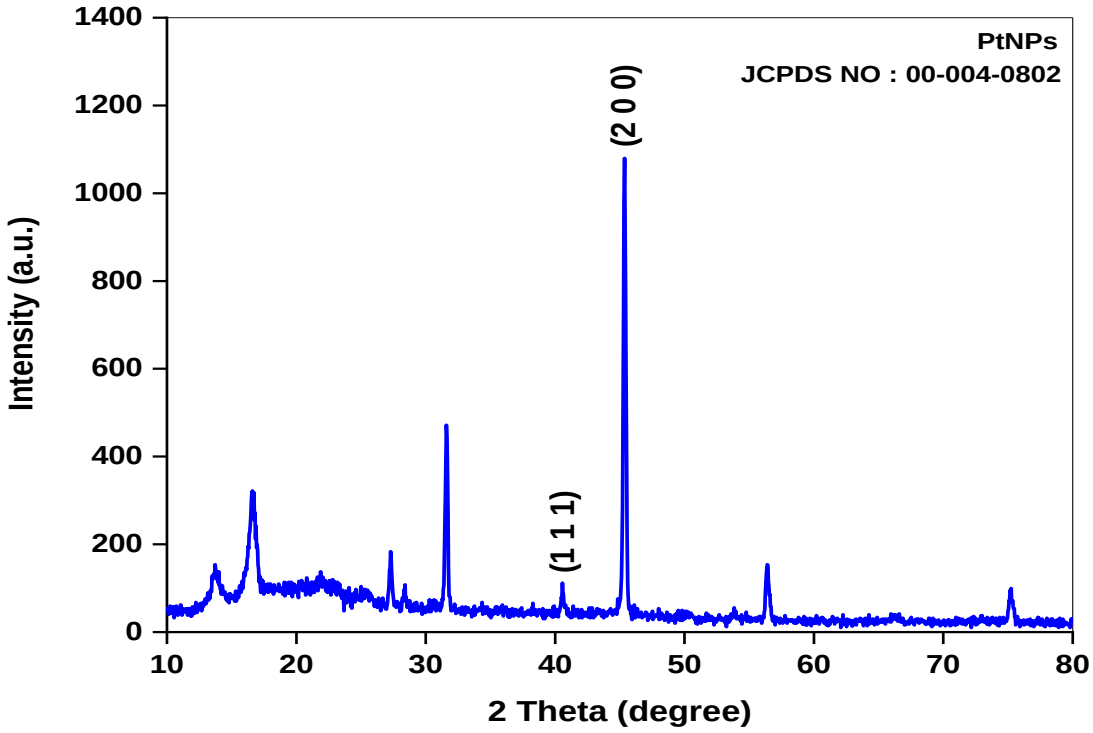


Figure(3.23): The size distribution of TEM for *S. Fradiae*(K).

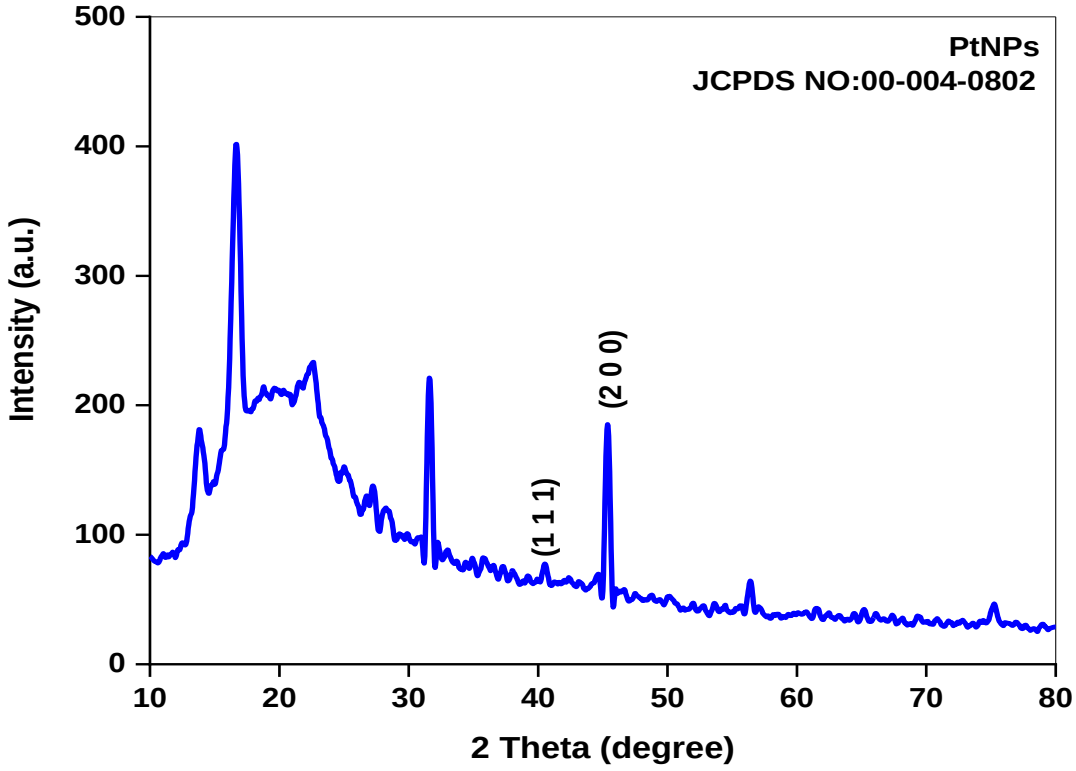
3.5.7 XRD Analysis

XRD carried out an analysis of the structure and crystalline size of the PtNPs (PtNPs). The XRD analysis of PtNPs biosynthesized by *Streptomyces Fradiae* showed that the peaks were observed at 2θ = 40.51, 45.39 corresponding to the lattice planes of the crystalline structure of PtNPs (111) (200), respectively; these outcomes are in agreement with the Joint Committee on Powder Diffraction Standards (JCPDS) file NO. 00-004-0802 . The XRD analysis of the biosynthesized PtNPs by *Streptomyces ziwulingensis* exhibited two peaks at 2θ = 41.31°, 44.39 ° corresponding to (111) (200) respectively. The XRD showed that the particles synthesized using the green method have a cubic crystal structure, other peaks were also observed before the corners, and a small percentage of shifting in the sample; the reason is that the samples contain other organic components, and as we mentioned earlier that the biosynthesis of nanoparticles has many disadvantages such as catalysts generation and difficulties in giving of better control of the distribution of size, shape,

and crystallinity (Borse *et al*., 2015). The result agreed with many studies like (Soundarrajan *et al*., 2012) and (Ali & Mohammed, 2021).



Figure(3.24): XRD analysis of PtNPs produced by *S. Fradiae*



figure(3.25): XRD analysis of PtNPs produced by *S. ziwulingensis*

3.6 Applications of platinum nanoparticles

3.6.1 Antibacterial activity

The antibacterial activity of biosynthesized PtNPs was studied against five species of pathogenic isolates bacteria from different sources (urine, wounds, burns, ear, blood). It was implemented using the disc agar diffusion method. The results showed that PtNPs potentiated Gram-positive and Gram-negative bacteria except for the bacteria of *Pseudomonas aeruginosa*; the highest inhibition zone was against *S. aureus* (18.2)mm, whereas the lowest was against *E. coli*(10.1)mm. See Table (3.3)and Figure (3.26). The antibacterial activity compares with the activity of antibiotics on these pathogenic bacteria, using Gentamicin in our study.

Table (3.3): Antibacterial activity of ptNPs Biosynthesized by *S. Fradiae* against pathogenic bacteria isolated from different clinical sources.

NO	Pathogenic bacteria	Inhibition zone (mm) of PtNPs	Inhibition zone (mm) of Gentamicin antibiotic
1	*Escherichia coli*	10.1	4.3
2	*Proteus mirabilis*	15.4	7
3	*Pseudomonas aeruginosa*	0	6.4
4	*Staphylococcus aureus*	18.2	7.3
5	*Acinetobacter baumannii*	10.3	4.6

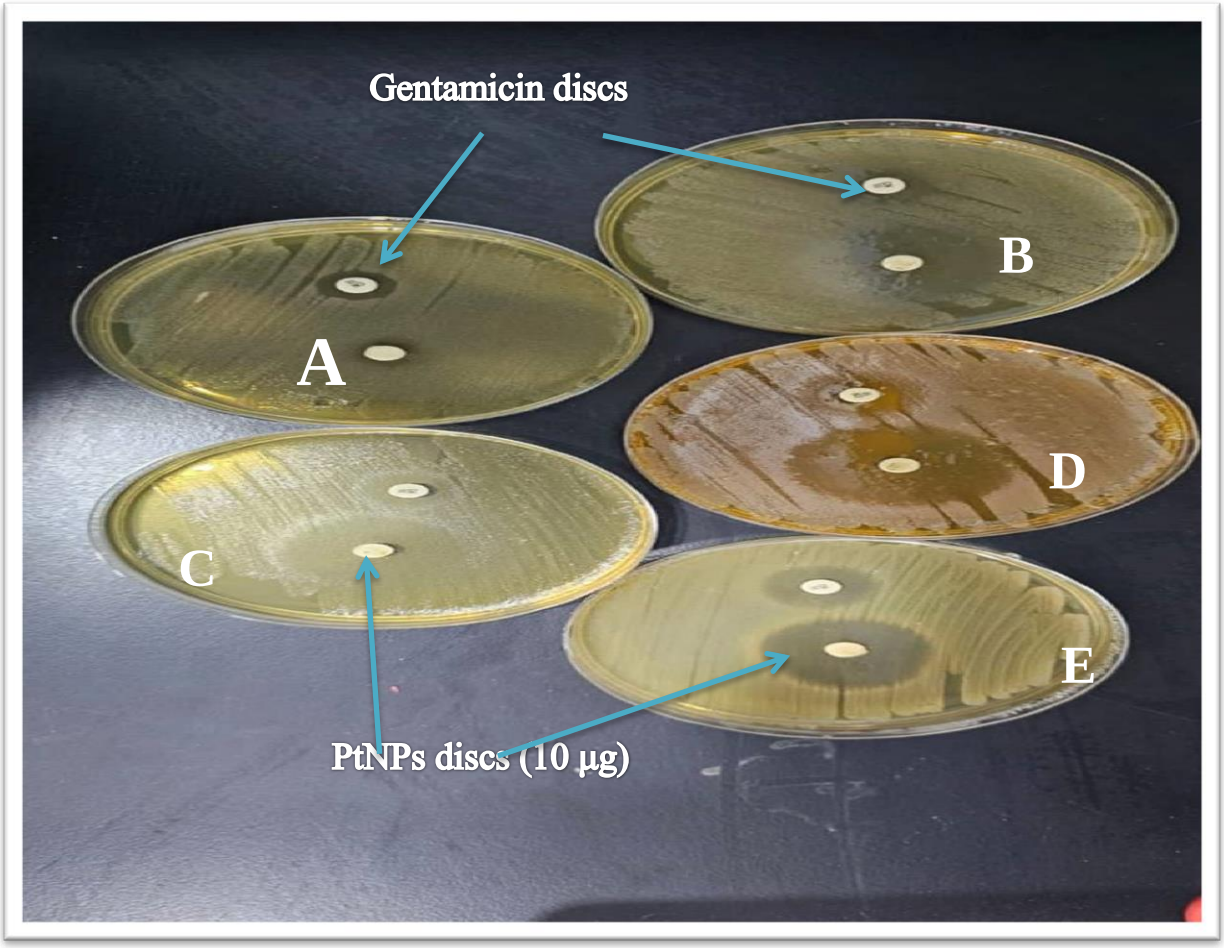


Figure (3.26): Antibacterial activity of PtNPs Biosynthesized by *S. Fradiae* against pathogenic bacteria and Gentamycin Antibiotic as a control, were: A: *p. aurogenosa, B: A. baumannii*, C: *E .coli*, D: *S. aureus, E: P. mirabilis*.

The results agreed with previous studies like (Thapa, 2017) and (Mie *et a*l., 2014). Our results proved the influential role of PtNPs in the inhibition process of pathogenic bacteria. Antibacterial activity findings revealed that PtNPs concentrations effectively suppressed the growth of pathogenic bacteria isolated from different sources. This activity may be due to the inhibition of translation and protein synthesis, membrane damage (Qing *et al.*, 2018; Yin *et al.*, 2020), high levels of ROS, and the release of metal ions by PtNPs. The results of the current study proved that inhibition of bacterial growth increased as the concentration of NPs increased (Fan *et al.*, 2021).

3.6.2 Cytotoxicity of PtNPs on cancer and regular cell lines

A test method (Freshney, 2021) was used to evaluate the toxicity of different concentrations of PtNPs against the human bone cancer cells (A -673), and normal mouse embryo MEF cell lines.

The cytotoxicity of PtNPs produced by *S. fradiae* and *S. ziwlunigenesis* were tested against A- 673 cell line at different concentrations (10,20,40,80) µg/ml for a period of 24 hrs at 37 C^0. The results showed that the toxicity of PtNPs increases with concentration. The highest inhibition ratio was (91% and 87.65%) at 80 µg/ml for *S. fradiae* and *S. ziwlunigenesis*, respectively Table (3.4) and (3.5). The results of this study agreed with (Zhou *et al*., 2019); this study provides an effective strategy for synthesizing bone-targeted nanoparticles with inherent anticancer potential for the treatment of malignant bone tumors. In addition, different concentrations of PtNPs (PtNPs) cause toxic effects on other cell lines (Abed *et al*., 2022; Bayat *et al*., 2023).

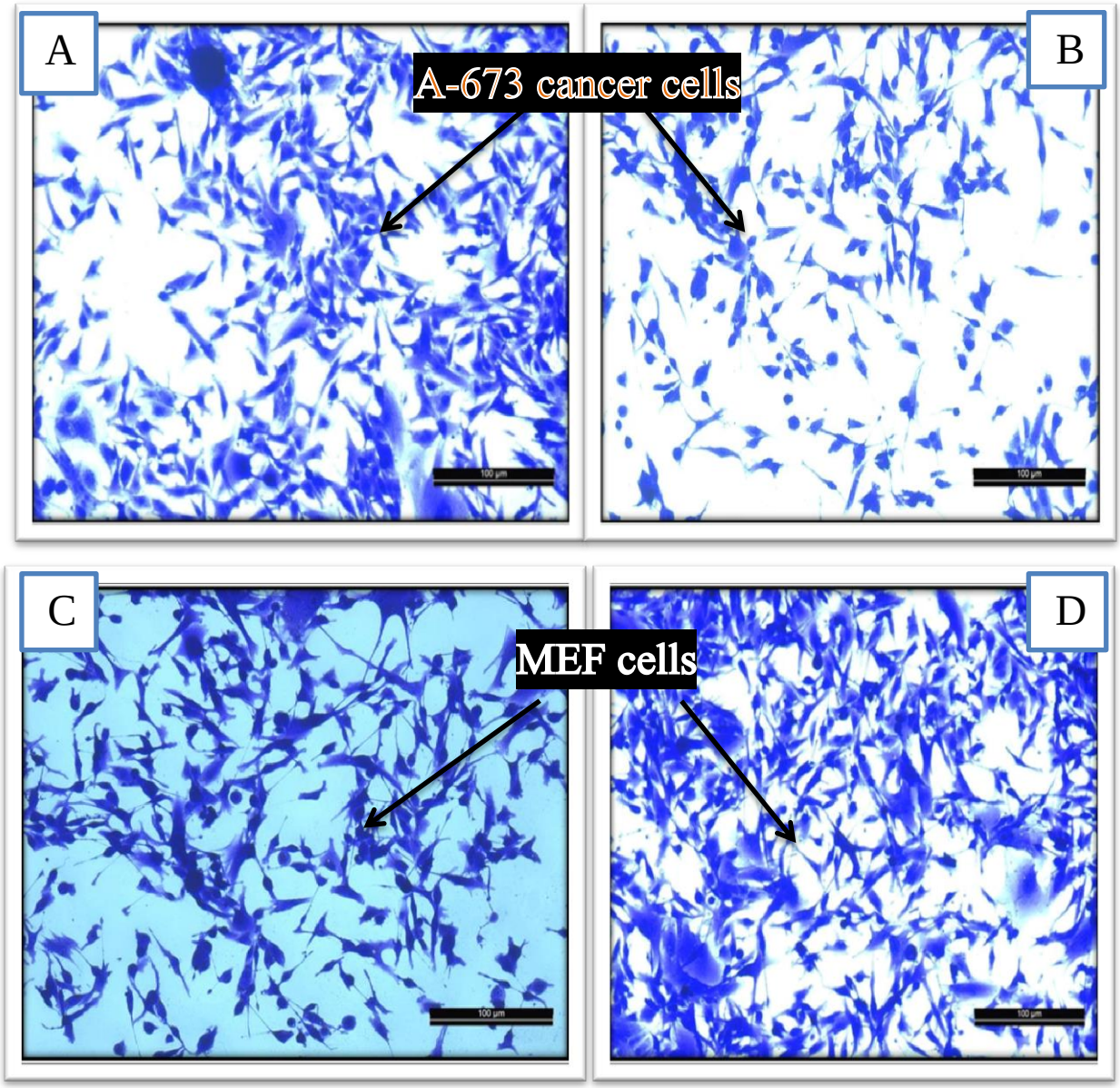
Table(3.4): Inhibition rates in the cancer cell line A-673 under the influence of different concentrations of nano k (PtNPs produced by *S. fradiae*) for 24 hours and exposure at 37°C for three replicates.

Standard deviation. inhibition ratio100%	Con . µg/ml
1.0 ± 15.36 d	**10**
1.2 ± 43.86 c	**20**
1.2 ± 77.02 b	**40**
1.3 ± 91.00 a	**80**

The letters in the same column indicate statistical differences at the (0.05≥*P*) level.

Table(3.5): Inhibition rates in the MEF cell line under the effect of different concentrations of Nano K for 24 hours and exposure at a temperature of 37°C for three replicates

Standard deviation. inhibition ratio100%	Con . µg/ml
1.0 ± 03.41 c	10
1.1 ± 13.28 b	20
1.1 ± 25.21 a	40
1.2 ± 29.12 a	80



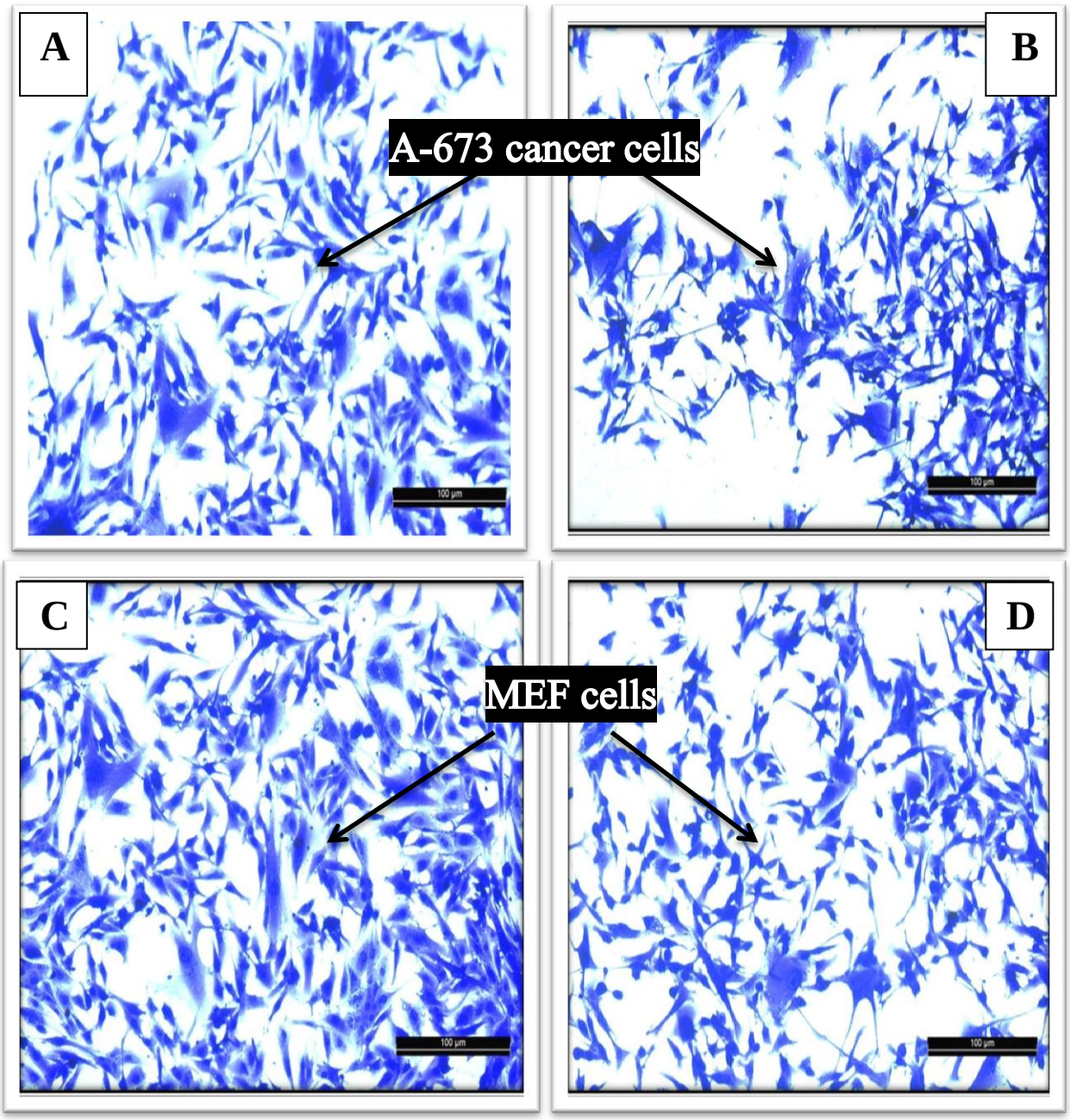
Figure(3.27): Cytotoxicity of PtNPs produced by *S. fradiae* on cancer and regular cell lines stained with crystal purple dye, A: A-673 cell line with 10 µg concentration of PtNPs (K), B: A-673 cell line with 80 µg concentration of PtNPs (K), C: MEF cell line with 10 µg concentration of PtNPs (K), D: MEF cell line with 80 µg concentration of PtNPs (K).

Table(3.6): Inhibition rates in the cancer cell line A-673 under the influence of different concentrations of nano H (PtNPs produced by *S. ziwlunigenesis* for 24 hours and exposure at 37 °C for three replicates.

Standard deviation. inhibition ratio100%	Con . µg/ml
1.0 ± 25.23 d	10
1.2 ± 63.20 c	20
1.3 ± 75.17 b	40
2.1 ± 87.65 a	80

Table(3.7): Inhibition rates in the MEF cell line under the effect of different concentrations of Nano H for 24 hours and exposure at a temperature of 37°C for three replicates

Standard deviation. inhibition ratio100%	Con . µg/ml
1.1 ± 11.90 b	10
1.2 ± 23.02 a	20
1.2 ± 25.85 a	40
1.3 ± 27.11 a	80



Figure(3.28): Cytotoxicity of PtNPs produced by *S. ziwlunigenesis* on cancer and regular cell lines stained with crystal purple dye, A: A-673 cell line with 10 µg concentration of PtNPs (H), B: A-673 cell line with 80 µg concentration of PtNPs (H), C: MEF cell line with 10 µg concentration of PtNPs (H), D: MEF cell line with 80 µg concentration of PtNPs (H).

The reported results indicate that the toxicity of biosynthesized PtNPs increases with increasing concentrations. It was observed that MEF cells (normal cells of mouse embryos) showed less toxic results compared to cancer cells when exposed to PtNPs. According to some hypotheses, NPs may interact with cancer cells in various

ways, including electrostatic interactions, capture by cell receptors, and ingestion of NPs by endocytosis. The ability of NPs to produce ROS inside cells may explain their cytotoxicity. ROS can cause mitochondrial malfunction, protein denaturation, DNA damage, and cell death.

The results of the present study agreed with (Zhou *et al*., 2019). This study provides an effective strategy for synthesizing bone-targeted nanoparticles with inherent anticancer potential for treating malignant bone tumors. In addition, different concentrations of PtNPs (PtNPs) cause toxic effects on other cell lines (Abed *et al*., 2022; Bayat *et al*., 2023). Many studies reregistered the activity of PtNPs in the cancer cell lines. Bendale & Paul (2017) proved the evaluation of the cytotoxic activity of PtNPs against normal and cancer cells and its anticancer potential through induction. PtNPs are relatively new agents that are being tested in cancer therapy. Their antioxidant effect, suppressing tumor growth, is most likely the cause. Targeting ligands can be added to increase the tumor-targeting capacity of functionalized metal PtNPs. Platinum itself has a strong anticancer effect. PtNPs act differently than platinum-containing compounds but possess similarly efficient anticancer activity. After entering the cell through passive diffusion or by endocytosis, the PtNPs utilize cytotoxicity depending on their size, concentration, and incubation time; this is mainly caused by the introduction of strand breaks in the chromosomal DNA DNA damage, leads to the inhibition of replication, and the induction of cell cycle arrest and apoptosis. Another possible mechanism of action of PtNPs involves inhibiting cellular metabolic activity, generating hydroxyl radicals, and releasing active free Pt2+ ions. This strategy is currently used in radiotherapy. At specific concentrations, PtNPs can also act as antioxidants (Prasek *et al*., 2013). Moreover, PtNPs can augment the host antitumor immune response through enhanced antigen presentation and T cell activation (Yu *et al*., 2022).

Chapter Four

Conclusions & Recommendations

Conclusions

In the present study, Soil was shown to be a rich source of Actinomycetes and bacteria, which were isolated and studied during our work. The results showed there were six isolates of Actinomycetes from bacteria they belong to *Streptomyces* sp. , one isolate was deposited in NCBI with unique GenBank accession numbers obtained for this sample to represent PP761597. The isolated Actinomycetes were used as a producing agent of extracellular biogenic platinum nanoparticles. UV, zeta potential, FESEM, FTIR, TEM, XRD, and AFM investigation confirm the elemental platinum and its crystalline structure. The present study provides a friendly, environmentally, and cost-effective approach for producing biosynthesized platinum nanoparticles used as antibacterial agents against pathogenic bacteria. Gram-negative and Gram-positive bacteria that cause diseases (pathogenic) are susceptible to platinum nanoparticles.. Platinum nanoparticles were significantly tested to be suppressing agents for A-673 bone cancer cell multiplication.

Recommendations

1. Increasing number of soil kinds and conditions, such as (aquatic environment, mountain area, and soils in extreme conditions), and at various depths.

2. Using organisms other than bacteria to produce biogenic PTNPs, such as fungi, algae, and other organism.

3. Biosynthesis of other metal nanoparticles such as gold, zinc, and copper.

4. Study the toxicity of PtNPs on experimental animals or Red blood cells.

5. Performing activity tests for platinum nanoparticles on some Dermatophytes.

6. Using other cancer cell lines to determine the antitumor activity of ptNPs, Such as PC3 and A375 cell lines.

Chapter Five

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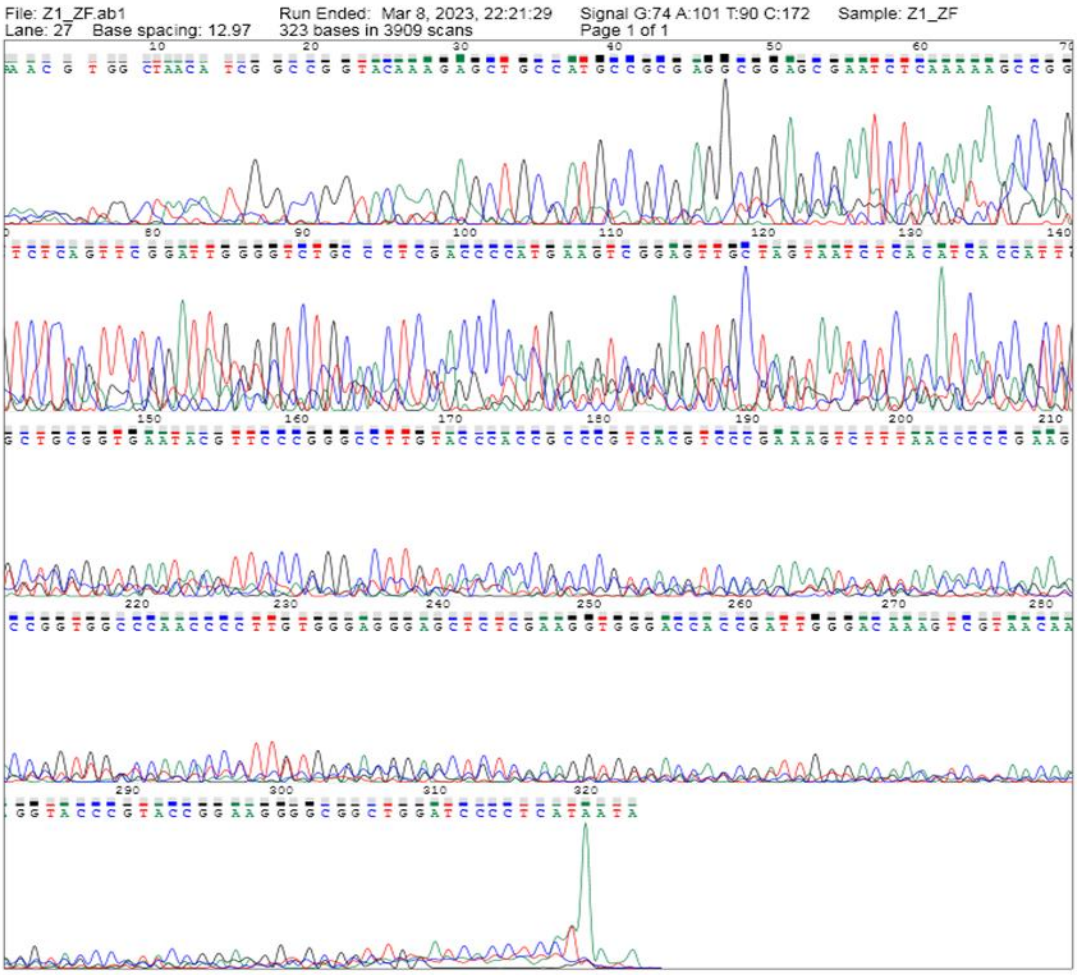
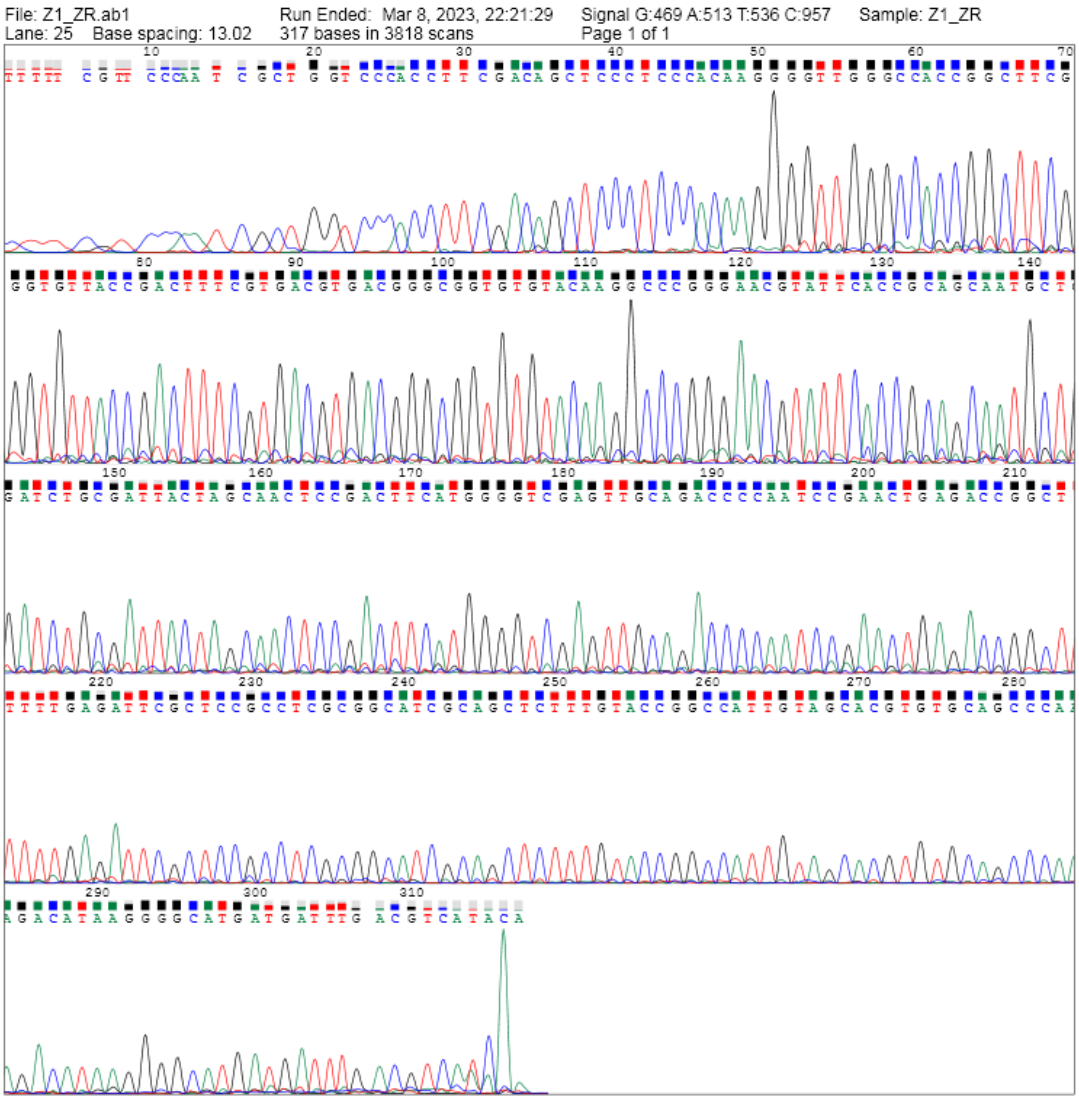
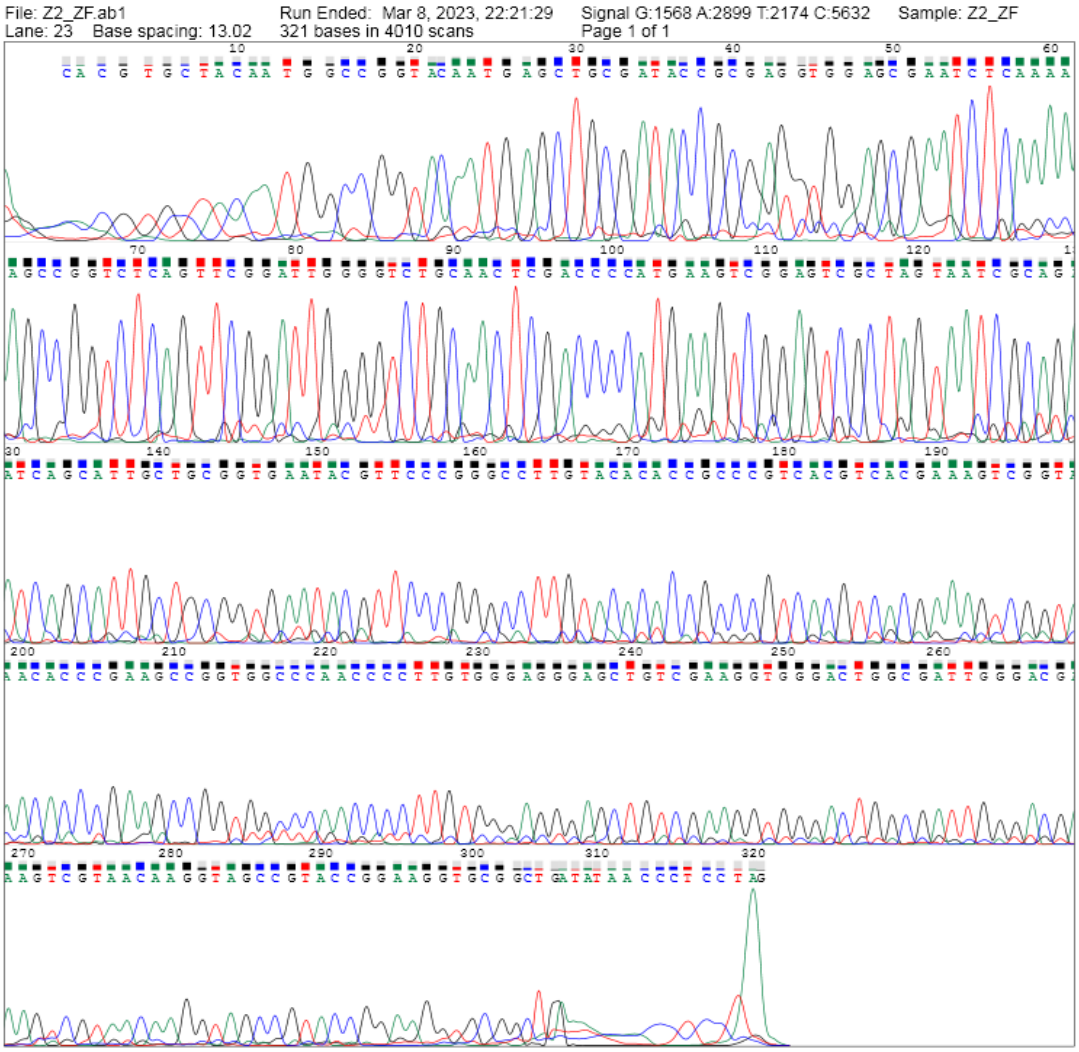
Appendix1: Identification of bacteria using the 16S rDNA gene : Z1: *Streptomyces omiyaensis* isolate, Forward strand.



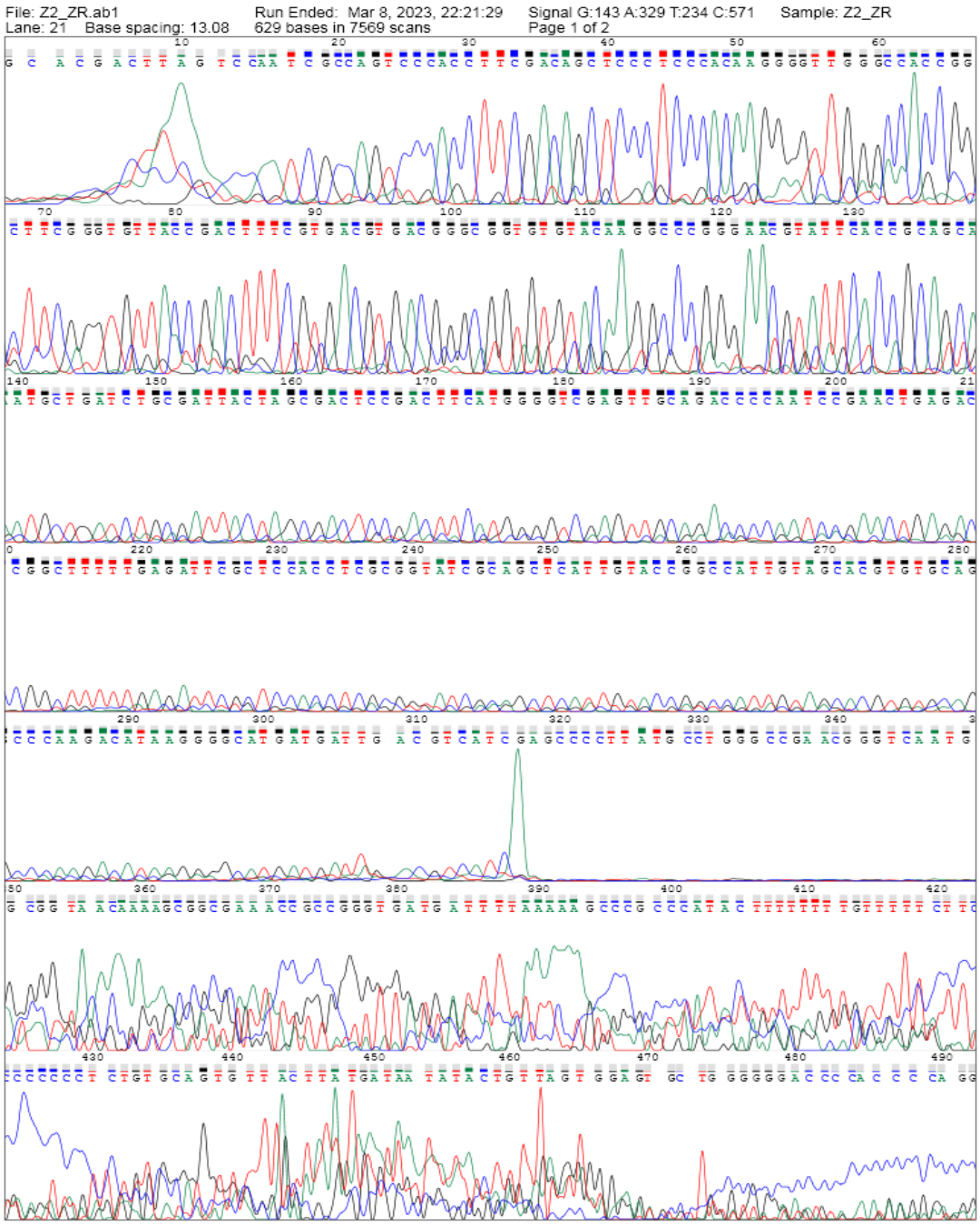
Z1 isolate, reverse strand



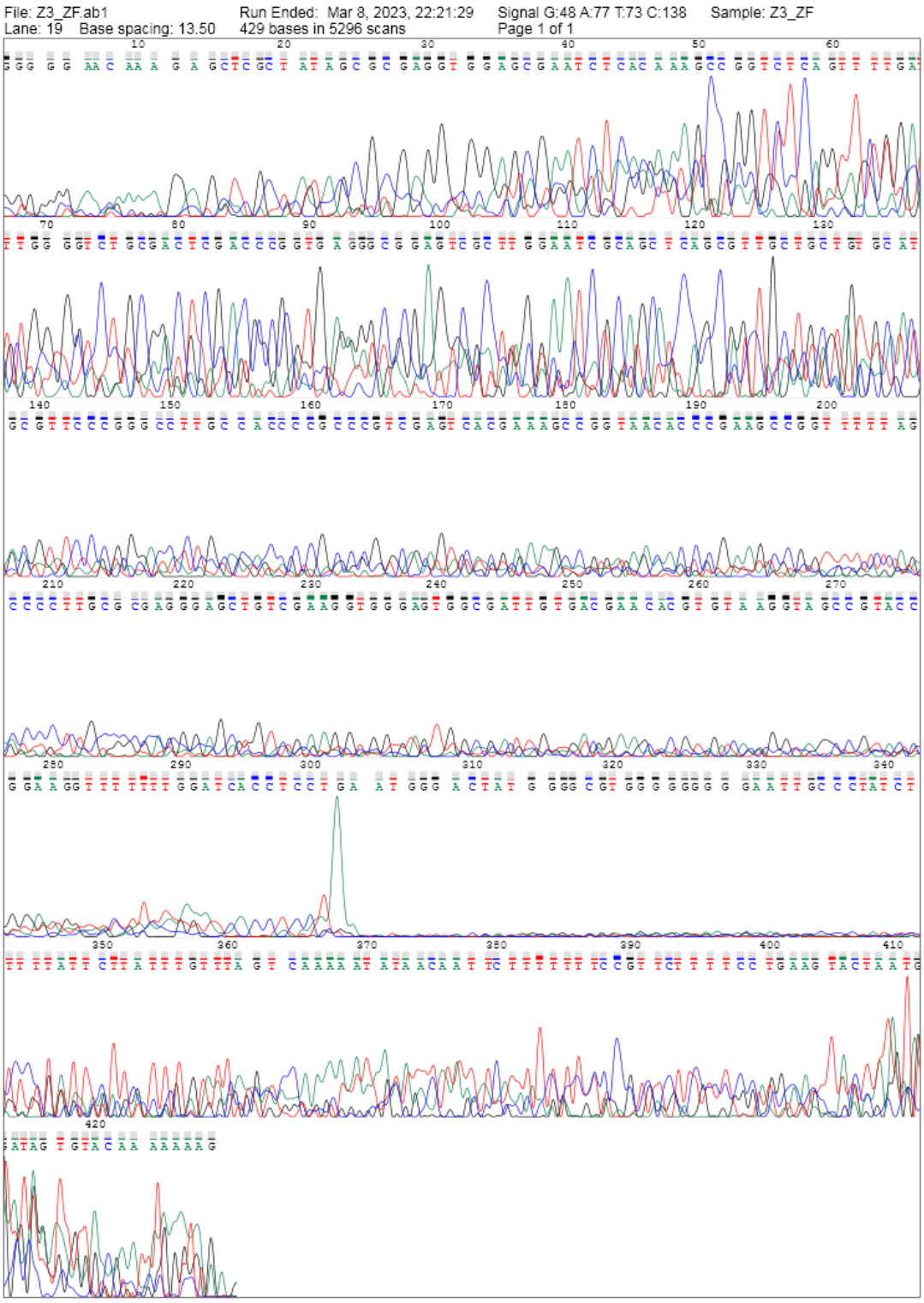
Z2 *Streptomyces macrosporeus* isolate, forward strand



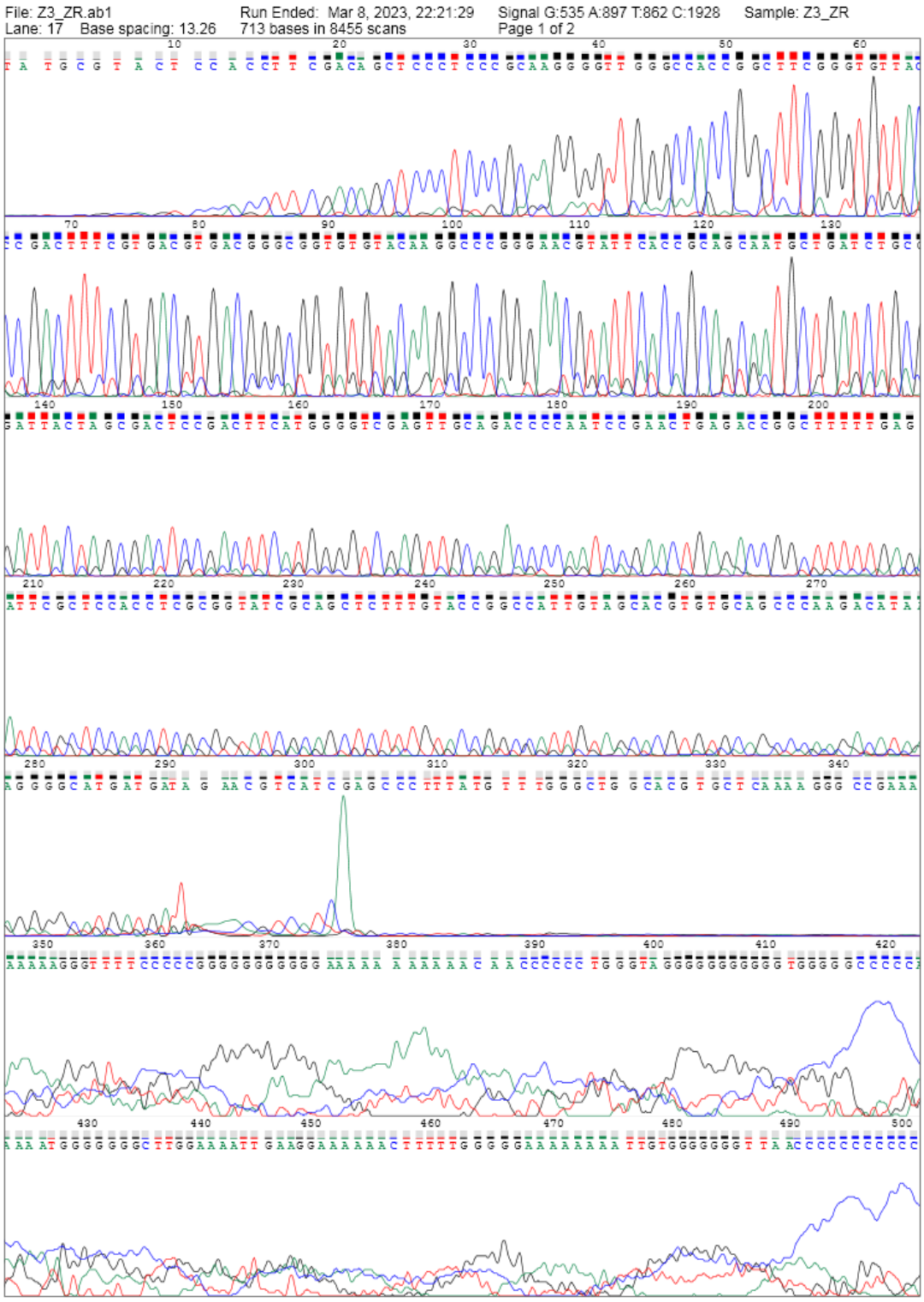
Z2 isolate , reverse strand



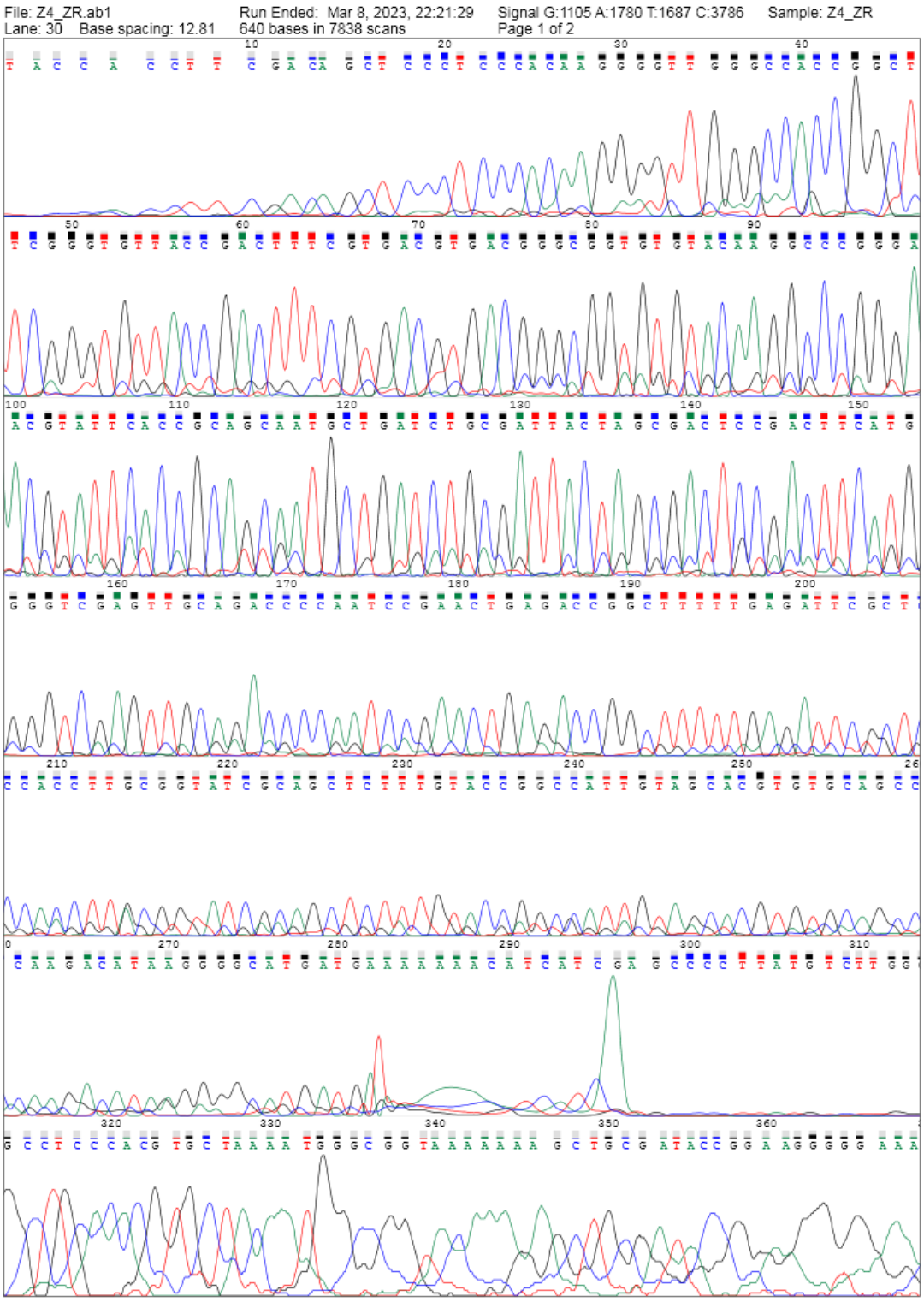
Z3 *Streptomyces thermolilacinus* isolate, forward strand



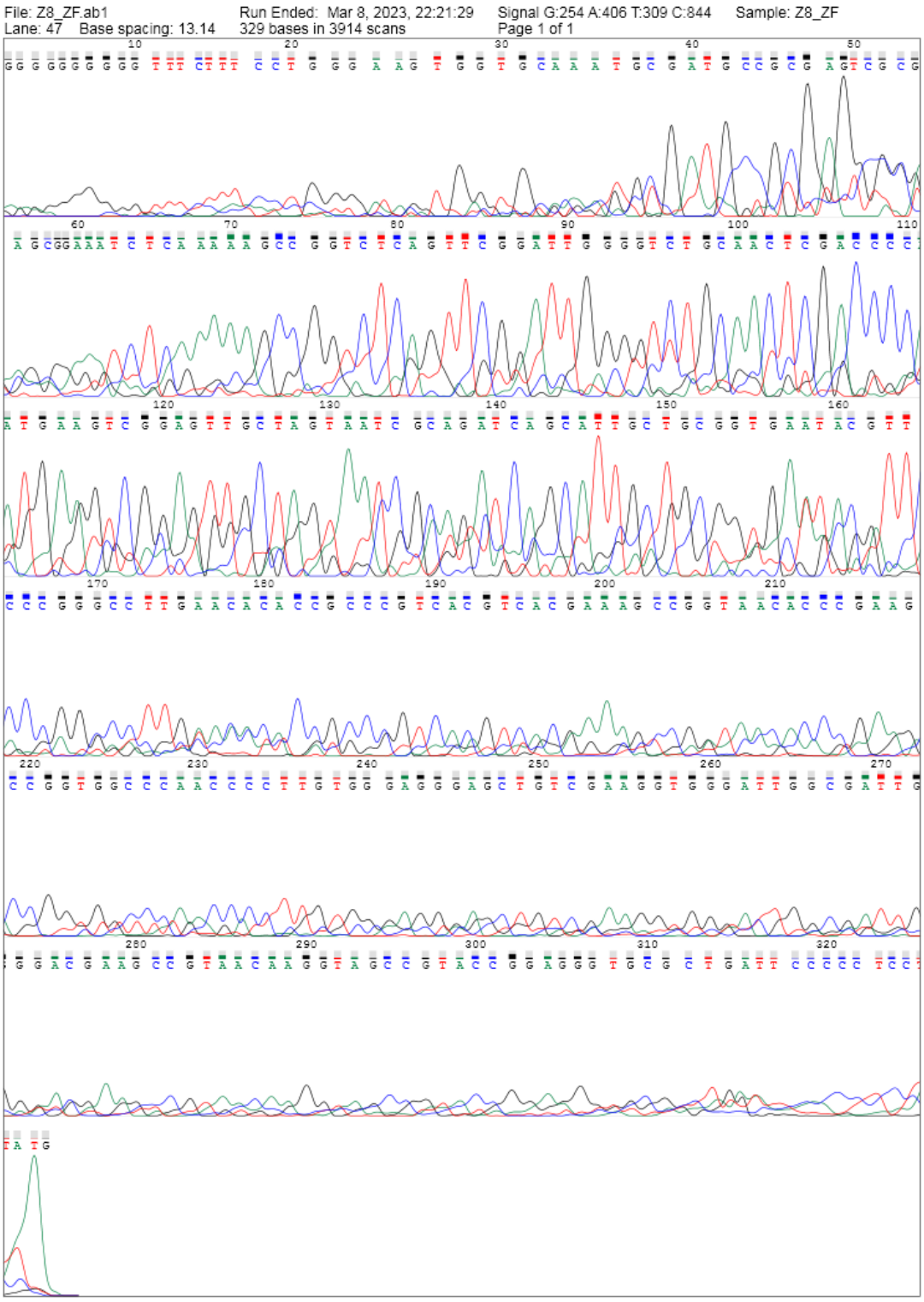
Z3 isolate, reverse strand



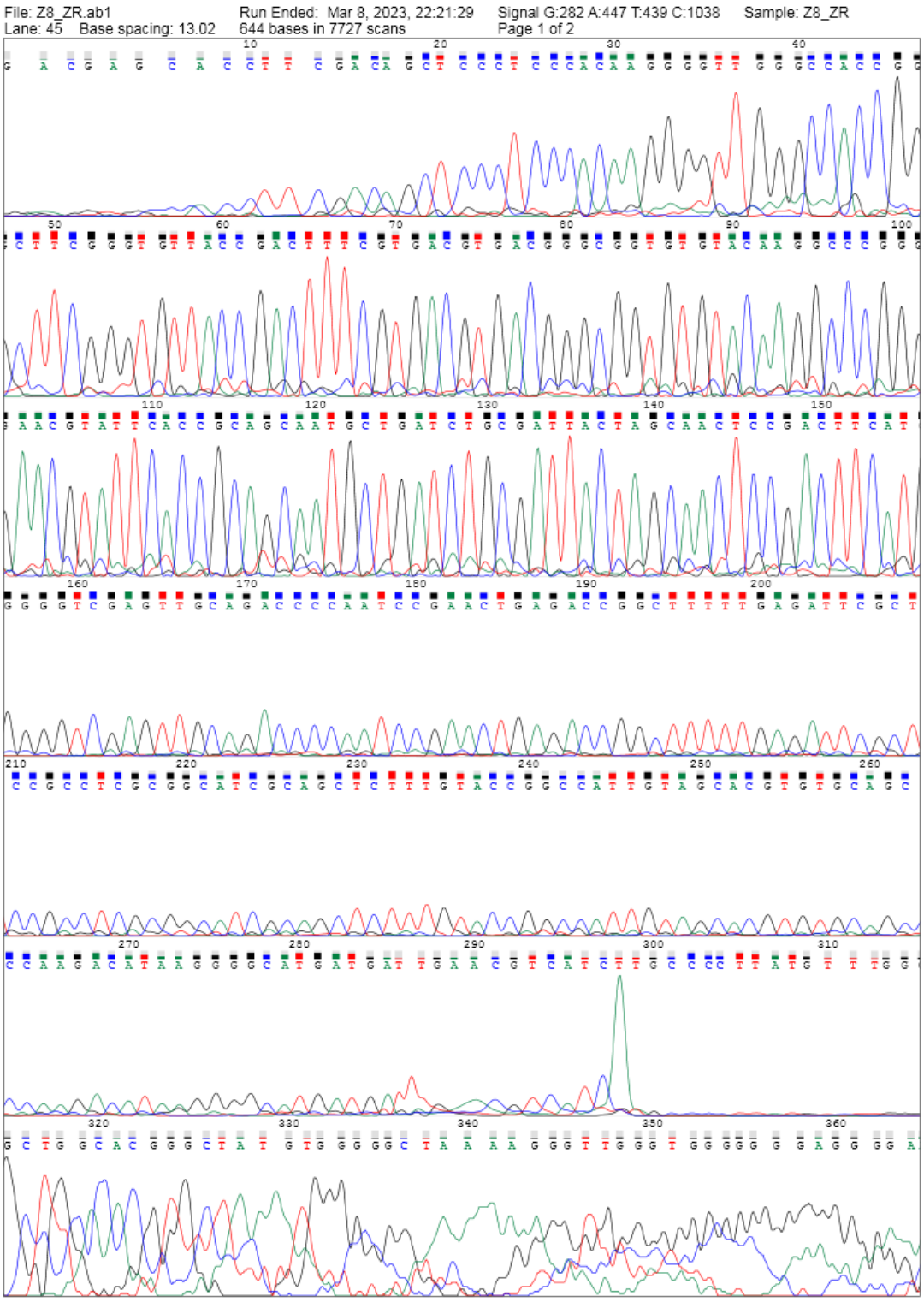
Z4 , *Streptomyces fradiae* isolate



Z8 *Streptomyces zaomyceticus* isolate, forward strand

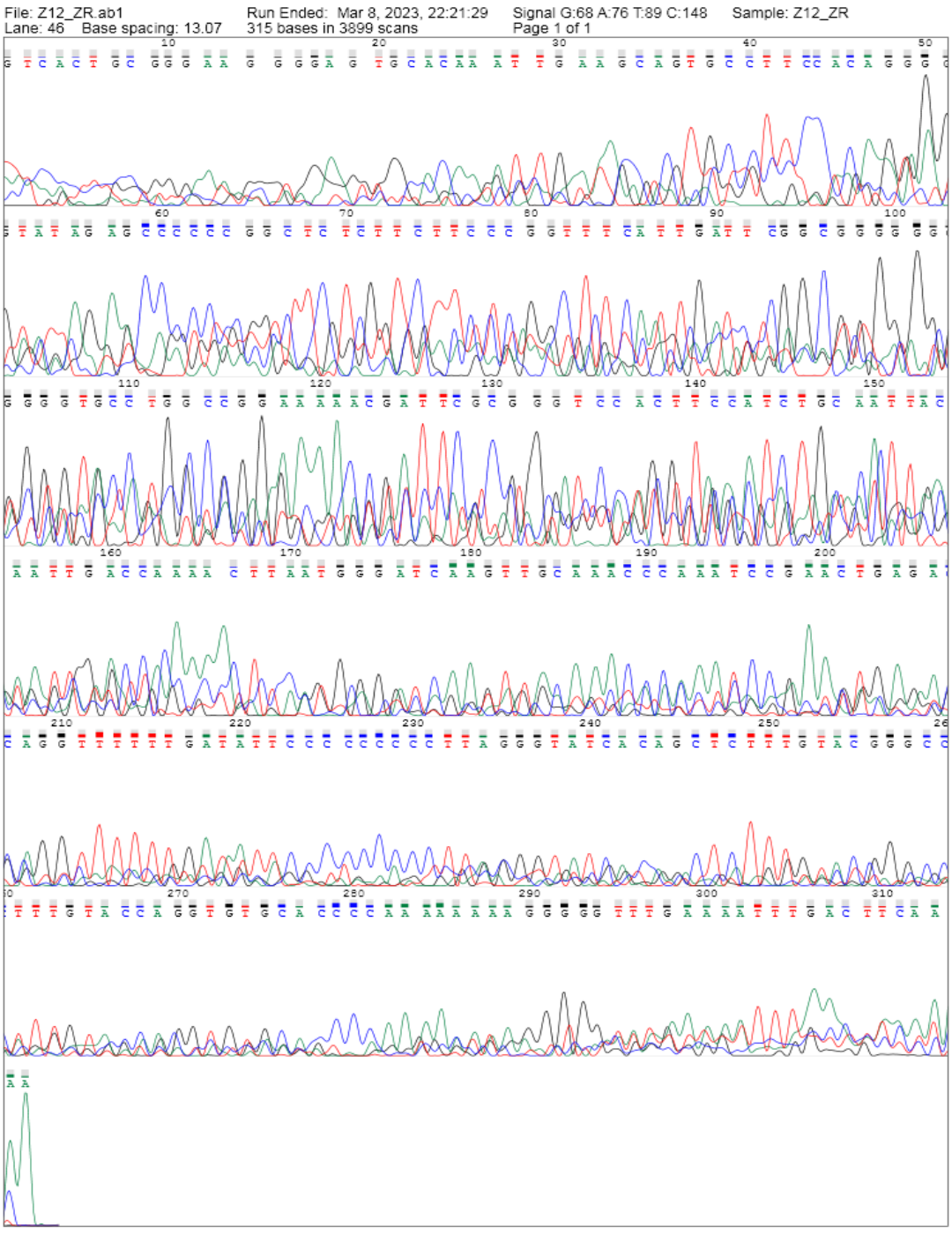


Z8 isolate , reverse strand

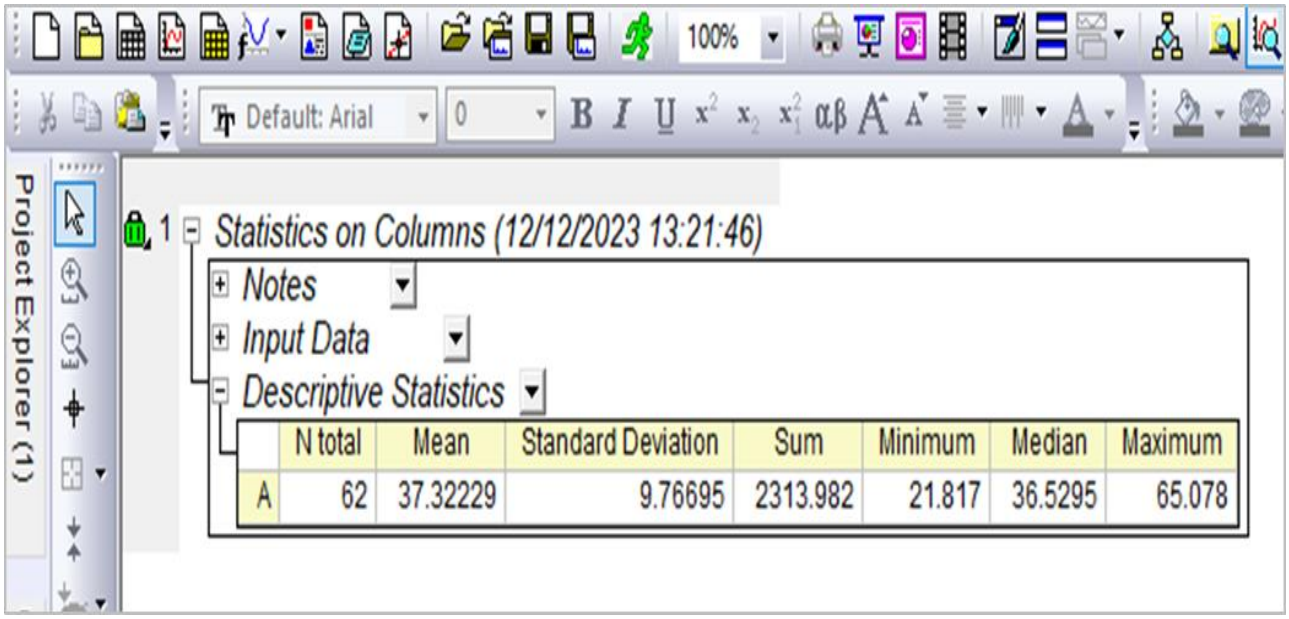


Z12 *Streptomyces ziwulingensis* isolate, forward strand

Z12 isolate, reverse strand



Appendix 2: Statistics of FESEM results for PtNPs biosynthesized by *S. zwiulengenesis*



Statistics on Columns (12/12/2023 13:21:46)

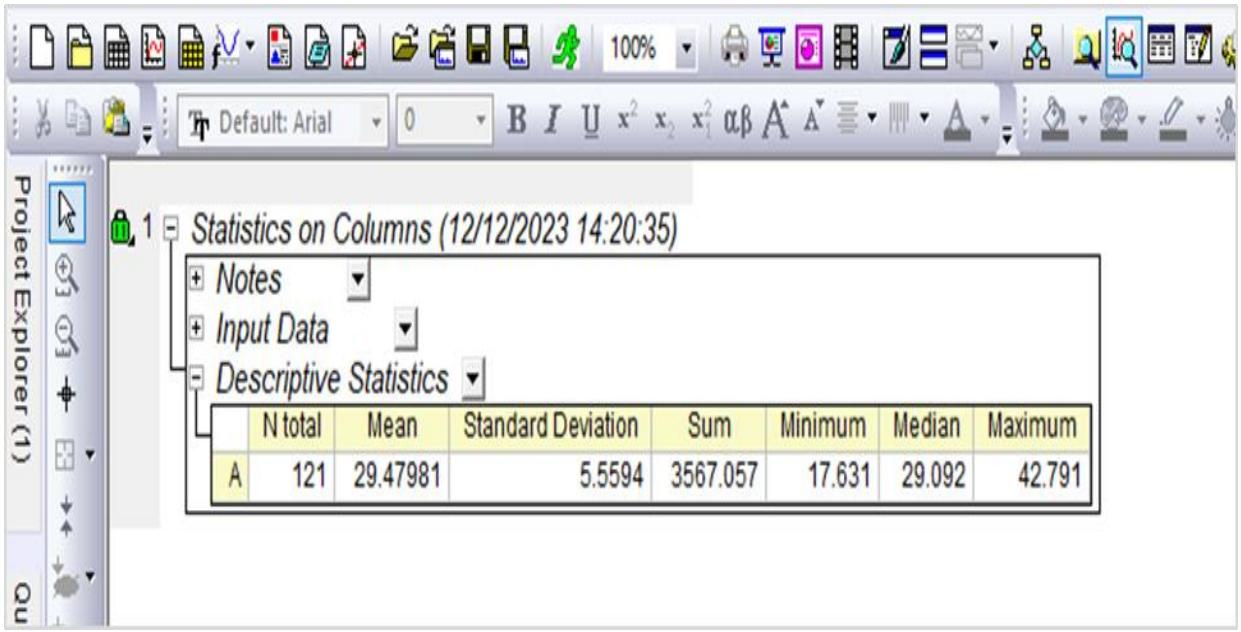
Notes

Input Data

Descriptive Statistics

	N total	Mean	Standard Deviation	Sum	Minimum	Median	Maximum
A	62	37.32229	9.76695	2313.982	21.817	36.5295	65.078

Appendix 3: Statistics of FESEM results for PtNPs biosynthesized by *S. fradiae*



Statistics on Columns (12/12/2023 14:20:35)

Notes

Input Data

Descriptive Statistics

	N total	Mean	Standard Deviation	Sum	Minimum	Median	Maximum
A	121	29.47981	5.5594	3567.057	17.631	29.092	42.791

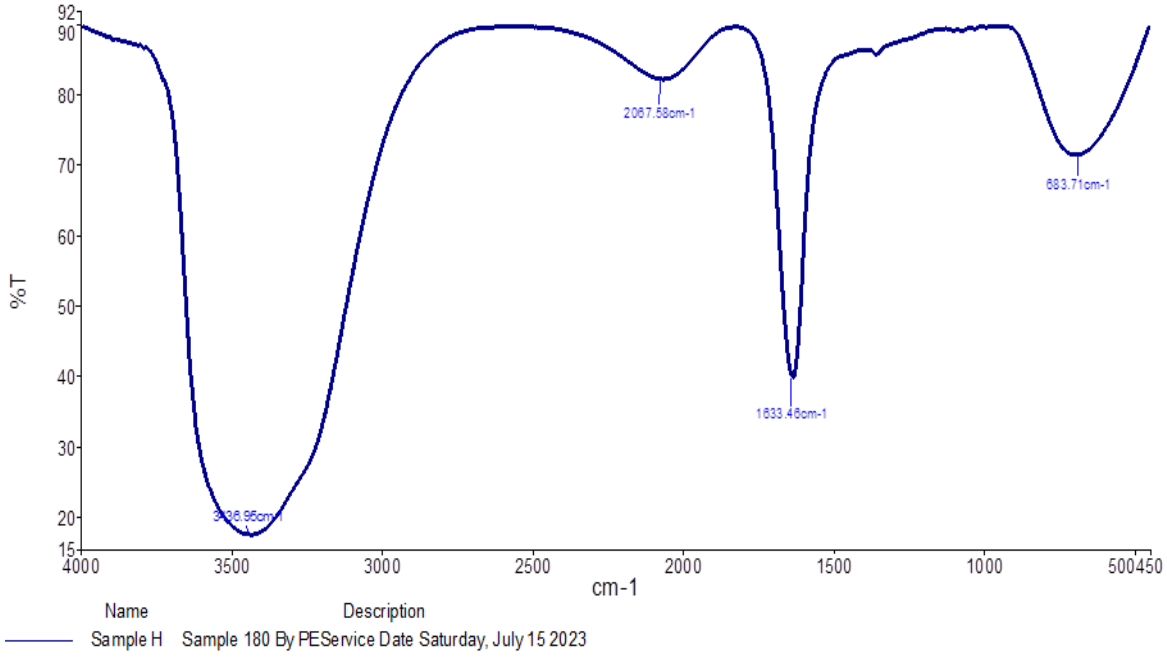
Appendix 4: Characteristics of the surface roughness parameter of PtNPs

Sample	Sq (nm)	Sp (nm)	Sv (nm)	Sz (nm)	Sa (nm)
H	39.78	155.8	132.7	288.5	28.69
K	7.81	31.79	36.79	68.68	6.15

Sq: Root mean square height, Sp: Maximum peak height, Sv: Maximum pit depth, Sz: Maximum height, Sa: Arithmetic mean height.

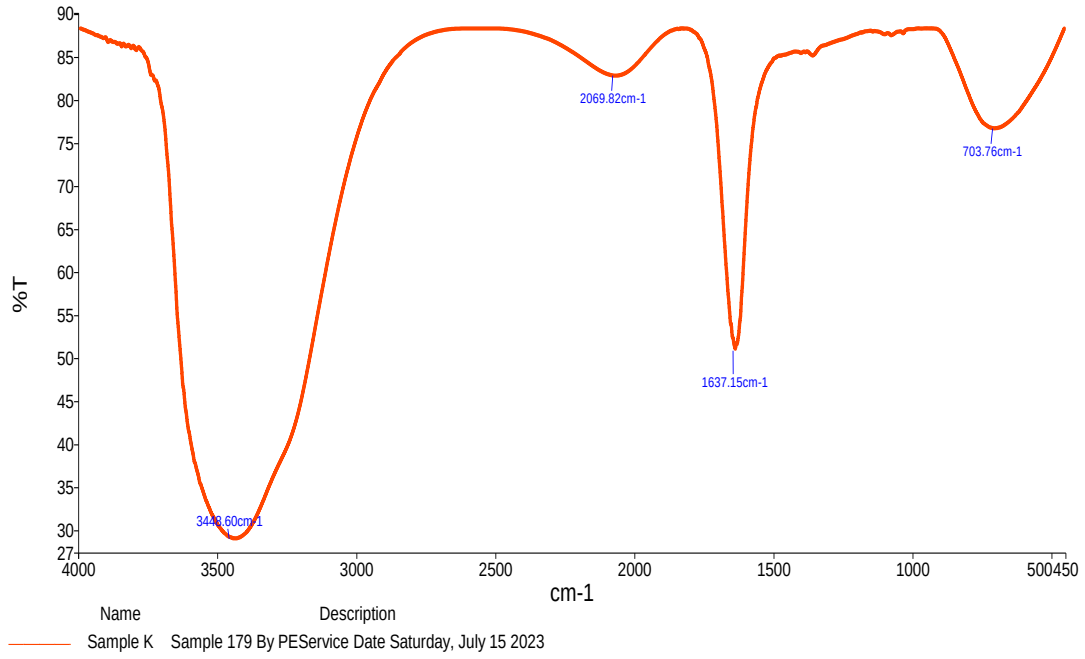
Appendix 5: FTIR analysis for PtNPs produced by *S. ziwulingensis*(H).

Peak Number	X (cm-1)	Y (%T)
1	3436.95	17.26
2	2067.58	82.46
3	1633.46	39.85
4	683.71	71.61



Appendix 6: FTIR analysis for PtNPs produced by *S. fradiae*(K).

Peak Number	X (cm-1)	Y (%T)
1	3448.60	28.91
2	2069.82	83.10
3	1637.15	51.11
4	703.76	76.93



الخلاصة

هدفت الدراسة الحالية الى تصنيع دقائق البلاتين النانوية بطريقة بيئية سهلة وصديقة ومنخفضة التكلفة باستخدام مستخلص البكتيريا المعزولة من التربة.

تقنية النانو الحيوية هي مجال متطور يتوافق مع التقدم التكنولوجي على المستوى الجزيئي أو الذري، حيث يتم من خلالها معالجة ودراسة الهياكل ذات مقياس الطول في حدود (1 - 100) نانومتر.

من خلال الدراسة الحالية تم جمع ثلاثين عينة تربة من مواقع مختلفة في محافظة ميسان للفترة من أكتوبر 2022 إلى ديسمبر 2022، بما في ذلك حقول الأرز والحدائق المنزلية والأراضي الزراعية. تم إحضار عينات التربة إلى مختبر التكنولوجيا الحيوية في كلية العلوم بجامعة ميسان. تم عزل البكتيريا باستخدام طريقة التخفيف التسلسلي. تمت زراعتها على وسط أجار النشا والكازين والنتريت وتم تنقيتها على أجار الجلوكوز المستخلص من الخميرة (وسائط انتقائية). تم التعرف على البكتيريا المعزولة باستخدام المجهر الضوئي، ثم تم تشخيصها عن طريق الفحوصات البيوكيميائية، وأخيراً عن طريق تفاعل البوليميراز المتسلسل (PCR) للتشخيص الجزيئي. بعد ذلك تم فحص العزلات البكتيرية لاختبار قدرتها على إنتاج جزيئات البلاتين النانوية. تمت طريقة العمل بخلط الراشح البكتيري مع محلول هيدرات حمض الكلوروبلاتينيك (H2pt2cl6). تم تأكيد تكوين الجسيمات النانوية البلاتينية الحيوية من خلال ملاحظة التغير اللوني والتوصيف الفيزيائي. تم فحص جسيمات البلاتين النانوية المصنعة حيويا للتأكد من سميتها ضد البكتيريا المسببة للأمراض، وخطوط الخلايا السرطانية، والخلايا الطبيعية.

أظهرت النتائج وجود ستة عزلات من بكتيريا Actinomycetes هي: *Streptomyces omiyaensis, Streptomyces macrosporeus, Streptomyces thermolilacinus, Streptomyces fradiae, Streptomyces zaomyceticus, Streptomyces ziwulingensis* تميزت العزلات باختلاف المستعمرة والشكل والصفات الأخرى. فيما يتعلق بالتشخيص الجزيئي، أظهر محرك NCBI BLASTn تشابهًا تسلسليًا كاملاً بنسبة 100٪ بين العينات الخمس (المعينة Z1 وZ2 وZ3 وZ4 وZ12) وتسلسل الحمض النووي لـ

Streptomyces sp.. في المقابل، أظهرت عينة واحدة (المعينة Z8) تشابهًا بنسبة 99٪ مع نفس الهدف. تم خلط الراشح البكتيري مع ملح البلاتين لإنتاج جزيئات البلاتين النانوية، وأظهرت النتائج تغير اللون لعزلتين بكتيريتين كمؤشر على إنتاج جزيئات البلاتين النانوية. أظهرت نتائج مقياس الطيف الضوئي المرئي فوق البنفسجي الذي كشف وجود مقياس الطيف الضوئي فوق البنفسجي لجسيمات البلاتين النانوية المصنعة حيويا باستخدام *Streptomyces fradiae* ذروة واسعة لهذه الجسيمات النانوية بطول موجي (362) نانومتر، في حين أظهرت جسيمات البلاتين النانوية المصنعة حيويا بواسطة *Streptomyces ziwulingensis* الطول الموجي (378) نانومتر. أظهرت نتائج المجهر الانتقالي الإلكتروني أن جسيمات البلاتين النانوية الحيوية في الدراسة الحالية يتراوح متوسط حجمها بين (10.83 - 29.19) نانومتر لبكتيريا *Streptomyces fradiae* و*Streptomyces ziwulingensis* على التوالي

أظهر المجهر الماسح بالانبعاث الميداني أيضًا فحص مورفولوجيا السطح وشكله وحجمه، حيث تم حساب متوسط قطر جسيمات البلاتين النانوية؛ كانت جزيئات البلاتين النانوية المنتجة باستخدام *Streptomyces ziwulingensis* كروية بحجم (21-65) نانومتر. وكان توزيع حجم الجسيمات 9.76 نانومتر، مما يؤكد أن معظم الجسيمات موجودة بين 21 و 65 نانومتر. وفي الوقت نفسه أكدت نتائج FESEM لجسيمات البلاتين النانوية المحضرة حيوياً باستخدام *Streptomyces fradiae* أن الجسيمات كانت كروية بحجم يتراوح بين (17-42) نانومتر، وكان التوزيع الحجمي 5.55 نانومتر. أظهرت نتائج مجهر القوة الذرية أن جسيمات البلاتين النانوية لها مساحات يتراوح قطرها من (11.35 – 1442 نانومتر) في *Streptomyces ziwulingensis* إلى متوسط قطر جسيمات البلاتين النانوية المنتجة باستخدام *Streptomyces fradiae* والتي كانت (41.66 نانومتر)، وكان حجم الجسيمات بين (4.89 - 227.1 نانومتر). أظهرت تحليلات زيتا المحتملة أن جسيمات البلاتين النانوية اكتسبت شحنات سطحية سالبة عند -28.6 ملي فولت و -38.1 ملي فولت. تُظهر بيانات التحليل الطيفي للأشعة تحت الحمراء لتحويل فوريه المجموعات المؤثرة التي قد تشارك في الاختزال والتخليق الحيوي للجسيمات النانوية البلاتينية. تم بواسطة XRD تحليل البنية والحجم البلوري للجسيمات النانوية البلاتينية.

تم اختبار السمية الخلوية لجسيمات البلاتين النانوية ضد خلايا سرطان العظام البشرية A-673 والخلايا الطبيعية لجنين MEF باستخدام تركيزات مختلفة (10، 20، 40، 80) ميكروغرام/مل؛ أظهرت البيانات أن هذه الجسيمات لها قدرة ضد الخلايا المكشوفة وكانت أعلى نسبة تثبيط (91% و 87.65%) عند 80 ميكروغرام/مل لكل من *Streptomyces fradiae* و *Streptomyces ziwlunigenesis* على التوالي. أكدت نتائج التحليل الإحصائي باستخدام برنامج Graph Pad Prism وجود اختلافات جوهرية بين التركيزات المثبطة للخلايا السرطانية عند مستوى معنوي <0.05. تمت دراسة النشاط المضاد للبكتيريا للـ PtNPs المخلق حيويا ضد خمسة أنواع من البكتيريا المسببة للأمراض المعزولة من مصادر مختلفة. أظهرت النتائج وجود تأثير قوي على البكتيريا الموجبة لصبغة جرام وسالبة الصبغة باستثناء بكتيريا *Pseudomonas aeruginosa*؛ أعلى منطقة تثبيط كانت ضد المكورات العنقودية الذهبية (18.2) ملم، في حين كانت أدنى منطقة تثبيط ضد *E. coli* (10.1) ملم.

جمهورية العراق
وز ارة التعليم العالي و البحث العلمي
جامعة ميسان
كلية العلوم – قسم علوم الحياة

التخليق الحيوي لجسميات البلاتين النانوية من بعض البكتريا الشعاعيه ودراسة فعاليتها الحيوية

رسالة مقدمة الى
كلية العلوم / جامعة ميسان جزء من متطلبات نيل شهادة الماجستير
في علوم الحياة

الطالبة
زينب ماهر مكطوف
بكالوريوس علوم حياة / جامعة البصرة
(2013)

بإشراف

الأستاذ المساعد الدكتور
رشيد رحيم حتيت

1445 هـ 2024 م