Republic of Iraq

Ministry of Higher Education

and Scientific Research

University of Misan

College of Science

Biosynthesis of Silver Nanoparticles from Some Actinomycetes and Soil Bacteria to Determination of Antibacterial and Anticancer Activities

A Thesis Submitted to

The Council of the College of Science / University of Misan In Partial Fulfillment of the Requirements for the Master Degree in Biology

By

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2022 A.D 1443 A.H


بِسْمِ اللَّـهِ الرَّحْمَـٰنِ الرَّحِيمِ
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الْبَرِّ وَالْبَحْرِ ۚ وَمَا تَسْقُطُ مِنْ
وَرَقَةٍ إِلَّا يَعْلَمُهَا وَلَا حَبَّةٍ فِي
ظُلُمَاتِ الْأَرْضِ وَلَا رَطْبٍ وَلَا
يَابِسٍ إِلَّا فِي كِتَابٍ مُبِينٍ ۞
صدق الله العلي العظيم
الأنعام(59)

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This is certify that this thesis entitled **(Biosynthesis of Silver Nanoparticles from Some Actinomycetes and Soil Bacteria to Determination of Antibacterial and Anticancer Activities)** was prepared under our supervision at the Department of Biology, College of Science, University of Misan, as partial requirement of the degree of Master in Biology.

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ACKNOLEDGEMENT

I am grateful to my supervisor Dr.**Rashid Rahim Hateet** who always made time to help me and motivated me to do my best by having ongoing and useful discussions and pointing me in the right direction.

I would like to thank the Biology department at the University of Misan for granting me permission and opening all research laboratory doors so that I could finish a large portion of my work, as well as Assist. Prof. Dr. **Maytham Abdulka dhim Dragh** the head of the Biology department, for always having an open door for me, and I would like to thank M.Sc **Shaima R. Banoon,** Biology department, college of Science, Misan University, for her help.

I would like to thank Dr. **Ali Taha Saleh** in Chemistry department, college of Science, Misan University, for his help in analysing chemical results.

DETECATION

TO MY LOVELY FAMILY, DAD, MUM, MY HUSBAND, MY BROTHERS, SISTERS AND MY SWEET KIDS .

Thuraya Mehbas Dewan

SUMMARY

Nanobiotechnology is a new field that emerged as a result of the merging of the nano technologywith biotechnology. Nanobiotechnologies have an advantage over non biological methods. According to this goal of the current research is to select and improve prospective strains from soil for extracellular biosynthesis of silver nanoparticles (AgNPs), as well as describe the AgNPs that created then determination the activity as antibacterial and anti tumor on breast cancer cell line. The isolation process began with experiment that tested soil samples, by collecting (40) samples from Misan cane field (because of the rich compound, Actinomycetes has been isolated from this soil in other research) and gardens for six months from (24November 2020 to 24May 2021). Then using the Microbiology Laboratory/ College of Science / University of Misan, to isolate the bacteria.

The isolated media was Starch-Casein-Nitrite Agar (SCN) agar and Nutrient agar, then purified on Yeast Extract Glucose Agar (YEGA) and nutrient agar. 16S rRNA gene sequencing is use to diagnose strains genotypically, which were then aligned and report in the NCBI. Some of these strains were new, so they are register at the Gene Bank. After that the Malt extract Glucose Yeast Extract Peptone Broth (MGYP) broth used for the synthesis of silver nanoparticles. To make AgNPs the best possible circumstances were used (26^{o}C for 7 days in shaker incubator, PH 7.0). Color change is the first indicate of AgNPs formation, depending on the synthesis strain. this color change because of the bio-reduction processes by reductase enzyme that reduce Ag^{+} to Ag^{0} and formation AgNPs. In this study the results shown a distinct optical absorption spectrum between (444 and 489 nm) wavelength of the synthesize AgNPs in the UV–visible spectrum. The produced particles are Spherical and generated crystalline indicating that they are face-centered

cubic (FCC) with intense peaks matching to the indexes of lattice planes of ((JCPDS), silver file No. (04–0783), according to X-ray diffraction (XRD) patterns meaning formation of silver metal. Silver nanoparticles with an average diameter of 50–100 nm well-dispersed and primarily spherical in shape , as shown in the Transmission Electron Microscopy (TEM) image. AgNPs were found to be reasonably spherical and uniform with diameter of 20 to 70 nm as shown in Scanning Electron Microscope. A quantitative examination of AgNPS using Energy Dispersive X-ray Spectroscopy (EDX) reveal very high and sharp silver peaks, indicating that the molecules appear on the surface of the AgNPs may have caused the reduction of silver ions to elemental silver. Atomic Force Microscopy (AFM) image analysis of AgNPs shown the highest average diameter manufactured by strain *M.lacticum* are 32.44 nm. Antibacterial and anticancer activity were examples of biological characterization of silver nano particles. Biogenic AgNPs have a strong antibacterial action against Gram-positive and Gram- negative pathogenic bacteria isolated from Al-Sadr hospital in Misan city (identified by VITEK2),to varying degree according to the types of AgNPs produced, the largest inhibition zone is 49 mm on *Staphylococcus hominis*, by AgNPs synthesize from *Pseudomonas mosselii*. The maximum antitumor activity of extracellular AgNPs on MCF7cell line (breast cancer cells) are record inhibition in growth at 100ug/ml with 81.73% and 81.22% of MCF7 Suppressed after 72 hours of incubation, by AgNPs synthesize using *M. lacticum* and *S. venesuelae* respectively. The lowest inhibition in growth was at 10ug/ml This indicates that as the concentration of AgNPs rises, so does its toxicity. The results shown that AgNPs strongly suppressed MCF-7 cell lines proliferation, with a The median inhibitory dose (IC50) value of 24.93ug/ml (sample T2) and 21.96ug/ml (sample T10). Thus, the in vitro biogenic silver nanoparticles showed the highest result as antitumor on MCF7 (breast cancer cells), and as antibacterial on pathogenic bacteria in our research.

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List of Abbreviations

Abbreviation	Key
AgNPs	Silver Nanoparticles
AFM	Atomic Force Microscope
bp	Base Pair
BLAST	Basic Local Alignment Search Tool
CLSI	Clinical and Laboratory Standards Institute
DMSO	Dimethyl Sulfoxide
EDX	Energy-Dispersive X-ray Spectroscopy
FCC	Face-Centered Cubic
MCF7	Michigan cancer Foundation-7
MGYP	Malt extract Glucose Yeast Extract Peptone Broth
MTT	Methyl Tetra Zolium
nm	Nanometer
NA	Nutrient agar
NCBI	National Center for Biotechnology Information
NPs	Nanoparticles
NUV	Near ultraviolet
PCR	Polymerase Chain Reaction
rpm	Rotation Per Minute
rRNA	Ribosomal Ribonucleic Acid
SCN	Starch-Casein-Nitrite
SEM	Scanning Electron Microscopy
SPR	Surface Plasmon Resonance Spectrum
ug	Microgram
ul	Microliter

TBE	Tris Borate EDTA
TEM	Transmission Electron Microscopy
UV rpm	Ultra-violate Rotation Per Minute
VITEK-2	Vitality Index of Traditional Environmental knowledge
XRD	X-ray Diffraction
YEGA	Yeast Extract Glucose Agar

CHAPTER ONE

INTRODUCTION & LITERATURE REVIEW

1.1 INTRODUCTION

Nanotechnology has recently revolutionized many fields of science in the quest for new ways to improve human living conditions, both in terms of health and environment (Staggers *et al.,* 2008; Hochella,2016; Lieber *et al.,* 2017). Nanoparticles were commonly used in materials science for a variety of applications because their morphological properties, scale, structure, and distribution can be regulated and guided in specific ways (Handy *et al.* ,2008;Parashar *et al.,* 2009; Anand *et al.,* 2017). However, since these methodologies are somewhat pricey, using this form of equipment in traditional laboratories is difficult. Furthermore, because of the chemical compounds used as reducing agents, the colloidal solutions produced may be hazardous to the environment,(Ahmad *et al.,*2004; Sajid *et al.,* 2015). As a result of the preceding, Since they use microorganisms, plant biomass, or plant extracts as reducing agents during chemical synthesis, the use of ecological and biological methods is a viable synthesis option for the manufacture of nanomaterials (Mohanpuria *et al.,* 2008; Espinosa *et al*., 2020). The analysis of ultra-small structures is known as nanotechnology The prefix "nano" derives from the Greek word "dwarf." "Nano" is a term used to describe a small amount of something. Nanotechnology is the transformation of single atoms, molecules, or compounds into structures in order to create materials and devices with unique properties .(Zinjarde,2012; Rai *et al*., 2012 ;Ogunsona *et al.,*2020). Nano technology is the creation , manipulation , and application of materials with a diameter of less than 100 nanometers (Malmsten, 2014). Nanoparticles are a unique class of materials with a wide range of applications, and they typically have a large surface area that is more chemically reactive than their fine structural equivalents (Mohanpuria *et al.,*2008),. Metallic NPs have several applications in biomedical domains, thus there is a lot of room for expansion in this field (Soltaninezhad *et al.,* 2015).

Nanotechnology is a modern multidisciplinary science that has an impact on the manufacturing, agricultural, and medical fields (Naito *et al.*,2018). The development of nanoparticles inspired by biology is ushering in a new age of nanotechnology (Dayma *et al*.,2019). Nanoparticles have their own set of characteristics, electrical, mechanical, magnetic, thermal, dielectric, optical, and biological properties. (Baranwal *et al.*,2018). Nanoparticles can be biosynthesized either intracellularly or extracellularly. Metal ions are reduced intracellularly on the surface of mycelia along with the cytoplasmic membrane, resulting in the development of nanoparticles in actinomycetes (Karthik *et al*.,2014). proposed a mechanism for silver nanoparticle production outside the cell in *Streptomyces* sp. LK3.The nitrate reductase enzyme is responsible for the conversion of nitrate to nitrite in the nitrogen cycle. (Golinska *et al.*, 2014) Silver nanoparticles have a variety of commercial uses, including antimicrobial, anticancer, optoelectronics, recording media, sensing instruments, catalysis, medicine, semi conductors, and so on. Via metal ion reduction (Nallamuthu *et al.*, 2012) , microorganisms play an important role in toxic metal remediation. The emergence of new bacteria resistant to current antibiotics has become a major public health concern, so there is a strong motivation to create new bactericides (Durán *et al.*,2007).

Aim of the study

The aim of this study is biosynthesize silver nanoparticles (AgNPs) and investigate their antibacterial and antitumor effects.

This aim is achieved using the following objectives:

1-Isolation and identification of Actinomycetes and bacteria from soil, and biosynthesize of silver nanoparticles (AgNPs) from them.

2- Characterization of biosynthesized AgNPs using physicochemical methods such us: UV-spectroscopy, XRD, SEM, TEM, EDX, AFM.

3- Study the antibacterial activity of the AgNPs against pathogenic bacteria isolation from different clinical cases by using conventional methods, VITEK 2 system, and evaluation the activity of AgNPs that synthesized as antitumor on MCF7 cell line (breast cancer cells).

1.2 LITERTURE REVIEW

1.2.1 Nanotechnology

The science and engineering involved in the synthesis, design, characterization, and application of materials and devices whose smallest functional organization in at least one dimension is on the nanometer scale can be described as nanotechnology (Mohanpuria *et al.,* 2008).Nanotechnology is a broad term that refers to any technology or research that acts at the nanoscale.

Nanoparticles have distinct qualities than larger particles, and these differences can be us ed in a variety of fields, including health, information technology, production of energy and storage, environmental applications, manufacturing and materials (Singh *et al.*,2008;Prabhu and Poulose, 2012; Yang, and Mai,2014), Figure (1.1).

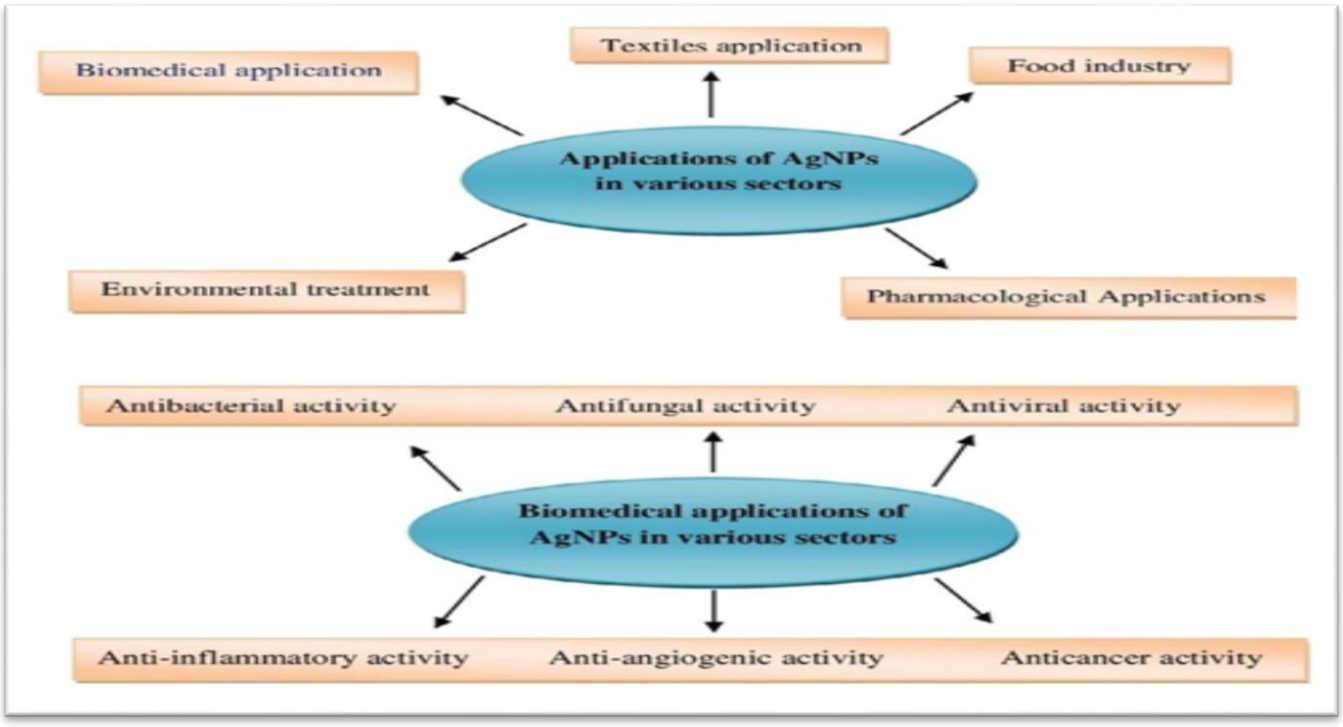


Figure 1.1: Application of Nanotechnology and Biomedical Application of AgNPs. (Verma and Maheshwari,2018)

Although nanoderive applications have enormous promise, there are some concern of regarding nanoparticles ability to harm human health and the environment. The various features that make nanoparticles so appealing are also properties that are likely to have an impact on ecosystems and species (Helland,2004). In his historic address "there's plenty of room at the bottom," physicist Professor Richard Feynman defined bionanotechnology as t he merging of biotechnology with nanotechnology for developing biological synthesis and environmentally friendly technology (Feynman,2018. Nanoparticles defined as clusters of atoms with a size of 1-100 nm and nano particles were gaining popularity in the twenty first century because they have well defined chemical , optical, and mechanical properties (Chan-Yeung, 2000).

Metallic nanoparticles are the most promising, since they have antibacterial characteristics because to their enormous surface area to volume ratio which is of interest to researchers due to rising microbial resistance to metal ions and antibiotics , as well as the creation of resistant strains (Rai *et al*,2009).

1.2.2 Biosynthesis

The emergence of resistant or even multiresistant pathogens has become a major issue, including *Staphylococcus aureus* resistance to methicillin and *Candida albicans* resistance to fluconazole (Schaller *et al.,* 2004),The introduction of a freshly developed wound dressing, on the other hand, has been a huge breakthrough in the care of wound sand infections. A new generation of dressing incorporating antimicrobial agents like silver was developed to prevent or reduce infection (Yin *et al.*,1999). It is well known that silver ions and silver based compounds are highly toxic to microorganisms (Duran *et al.,* 2007). Because of its cost effectiveness, low toxicity, and superior optimizing control, the biologic route of synthesis employing bacteria, yeast, fungi, algae, and plants is gaining popularity (Golinska *et al.*,2014). Microorganisms synthesize nanoparticles via both extra cellular and

intracellular pathways (Duhan, and Gahlawat 2015). Extracellular biosynthesis is preferred to intracellular biosynthesis because it requires less sophisticated downstream processing , while intracellular requires ultra sonicator for cell lysis to purified synthesize nanoparticles (Durán *et al.*,2011).The slow rate of synthesis, non uniform size of nanoparticles, and polydispersity are all factors that limit bacterio genic nanoparticle synthesis (Zhanga *et al.*, 2013). Further more , there are no optimization studies of various factors such as silver nitrate (AgNO3) concentration , pH, or temperature for increased and quick AgNPs synthesis (Rajora *et al.*, 2016).

1.2.3 Nanoparticles

Nanoparticles are defined as particles with more than one dimension on the order of 100 nm or less and are regarded the building blocks of nanotechnology (Pitkethy,2003) ,figure (1.2). Nano structured materials are being offered for use in communications, medical, transportation, agriculture, and other industries as better-built, long-lasting, cleaner, safer, and smarter productes. Researchers from both biological and engineering sectors were interested in molecular recognition, biomolecule nanocrystal conjugates as fluorescent labels for biological cells, and DNA mediate groupings of nanocrystals (Zhang *et al.*, 2016). Nanotechnology encompasses a wide range of disciplines, from biology to material science, physics to chemistry, and other specialties. The unique qualities of nanotechnology are governed by the controlled size, shape, composition, crystallinity, and structure dependent properties of NPs. The regulated biogenesis of nanoparticles is of great scientific and technological interest because microorganisms collect target ions from their surroundings and then use an enzyme mechanism generated (Intra/Extra) Cell activities to convert the metal ions into the element metal (Singh *et al.*,2015).

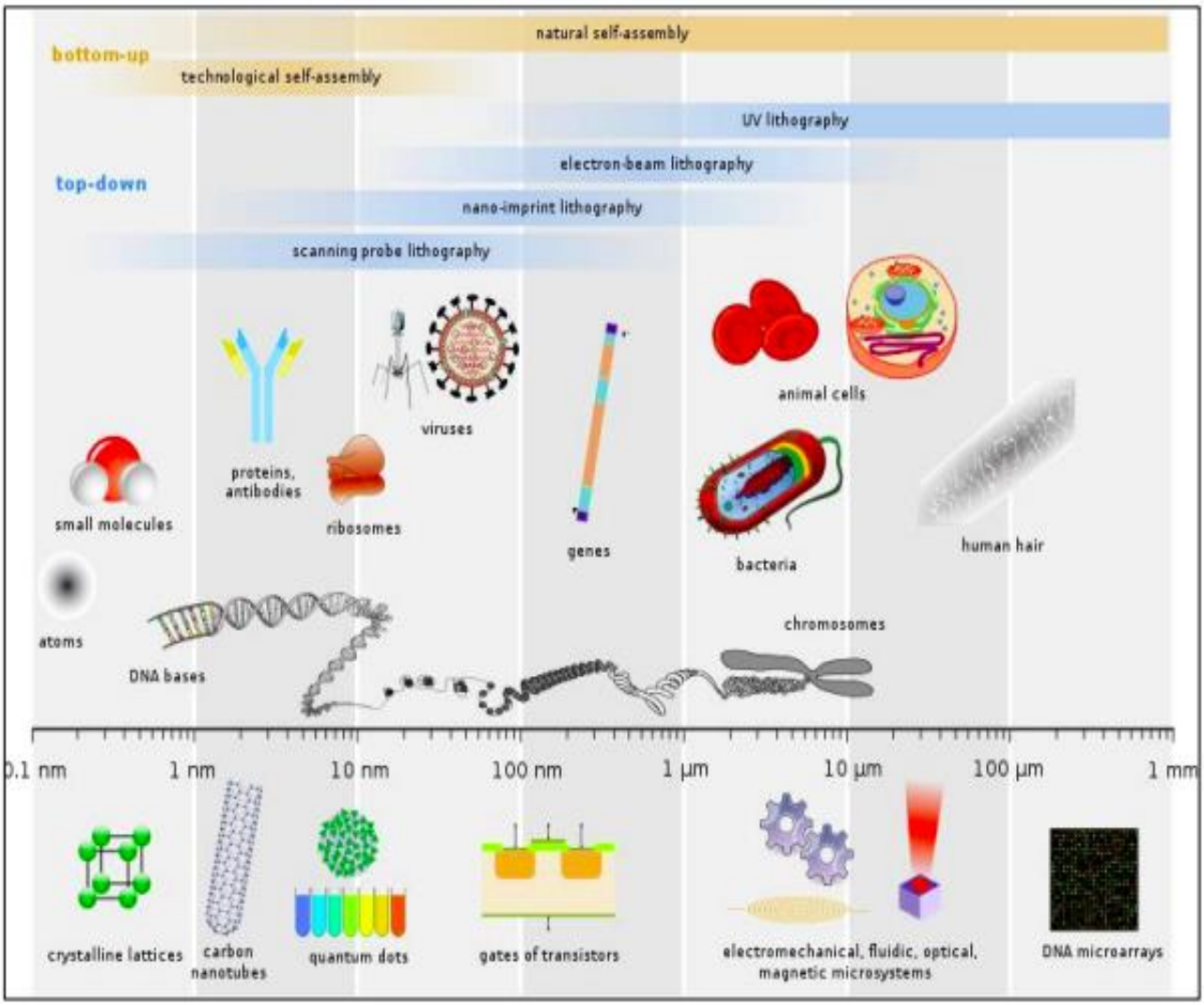


Figure 1.2: Illustration of Nanoscale (Paumier,2008).

The synthesis and application of Nanoparticles in biology is one of the most significant advances in Nanotechnology. Because of their unusual physical properties, chemical reactivity, and possible applications in catalysis and biological labeling, drug delivery, antibacterial activity, antiviral activity, gene therapy, detection of genetic disorders, and DNA sequencing (Govindaraju *et al.*,2008). A large volume of dangerous and unnecessary chemicals, substances, is causing disturbing environmental waste, which is

causing harm to our ecosystem. As a result, our need to learn about nature and its products has led to the creation of Biomimetic approaches for the advancement of Nanoparticle synthesis processes (Thangaraju *et al.*,2012).

Nanoparticles are an excellent platform for cancer targeting and diagnostics. In most cases, less than 10% of all NPs delivered systemically end up in the tumor. Blood component interactions with nanoparticles are described, as well as how these interactions influence sol id tumor targeting. Serum proteins adsorb into nanoparticles in the blood to form a protein corona,which is reliant on the physicochemical features of the nanoparticles.

These serum proteins can prevent nanoparticle tumor targeting ligands from attaching to the receptors on tumor cells. Furthermore, serum proteins can induce macrophages to take up nanoparticles, reducing nanoparticle availability in the circulation and limits accumulation of the tumor. The creation of this protein corona also increase the hydrodynamic size of nanoparticles or cause aggregation, making larger nanoparticles can't enter through the pore of the leaky vessels of the tumor. Recent research has focused on Lazar ovits J, establishing new chemical techniques to decrease or remove serum protein adsorption and restore nanoparticles' tumor cell targeting capacity. Designing NPs with appropriate physicochemical characteristics and high tumor accumulation requires a thorough under standing of nanoparticle-blood interactions(Lazar ovits *et al.*,2015).

1.2.4 Preparation Method of Nanostructures

There is a need to improve environmentally friendly nanoparticle synthesis procedures. For the synthesis of metal nanoparticles, two basic methods have been proposed: "bottom- up" and "top-down. "The "top-down approach" deals with the fabrication of nanoparticles by directing their assembly with bulk material (larger ones) figure (1.3). By beginning at the molecular level and retaining precise control of

molecular structure, the "bottom-up approach" is responsible for the construction of larger and more complex structures,(Pattekari *et al*., 2011).

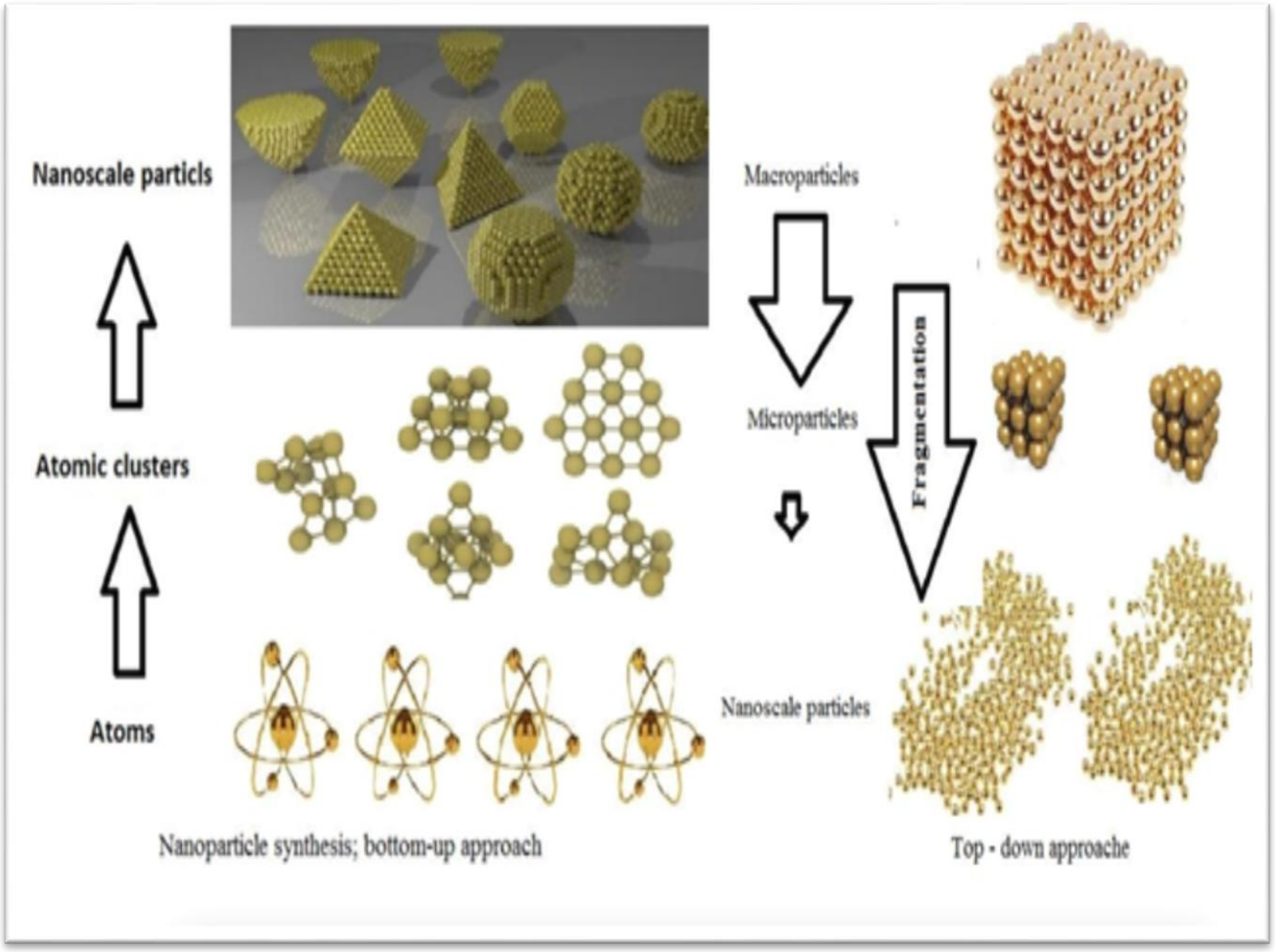


Figure1.3: Nanoparticle Synthesis: Bottom-up and Top-Down Approach (Hassan,2018).

Both of the basic approaches for the synthesis of nanoparticles described above were accomplished using various physical, chemical, and biological methods. Metal NPs were usually synthesized using physical and chemical methods, due to their monodisperse nanoparticle shape and specificity Various methods have been used for the synthesis of metal NPs. Now-days, biological methods are gaining ground due to the disadvantages of physical and chemical methods, such as involvement of hazardous chemicals, use of

different kinds of radiations, and most important is their high cost used for synthesis metal nanoparticles. Biological approaches are gaining traction these days due to the drawbacks of physical and chemical procedures, such as the use of toxic substances, the use of various types of radiation and most importantly, their expensive cost (Ingle *et al*., 2008).

1.2.5 Nano Silvers and their Mechanisms

Silver has long been known to be harmful to a wide range of microorganisms (Liau *et al*.,1997), silver based compounds have been widely used in a variety of bactericidal purposes. Several silver salts and derivatives are used commercially as antibacterial agents. Ag^+ have a well-known bactericidal action on microorganisms, however the bactericidal process is only partially understood. It's been proposed that Ag^+ interacts significantly with the thiol groups of essential enzymes, rendering them inactive (Gupta and Silver, 1998) Experiments show that when bacteria were exposed to silver ions, their DNA loses its capacity to replicate. Other research has found indications of structural alterations in cell membranes as well as the creation of tiny electron dense granules from silver and sulfur(Feng *et al*., 2000;Hallol,2013). Noble metal NPs , such as (AgNPs) , can efficiently fight Gram-negative and Gram positive bacteria (Huang *et al*.,1996; Shenashen *et al*.,2014) via six antimicrobial path ways,(Li, Q. Mahendra *et al*.,2008; Hussain,2005):

(i)Cell wall and peptidoglycan distraction.(ii) toxic ions release. (iii) protons efflux bombs distraction and membrane charges modification. (iv) reactive oxygen species (ROS) formation and cell wall degrading. (v) DNA, RNA, and proteins degrading by (ROS). (vi) low production of adenosine triphosphate ATP (Nguyen *et al*.,,2019). Because of its small particle size and large specific surface area, silver nanoparticles promotes faster ion dissolution than equivalent bulk materials, as shown in figure (1.4), potentially increasing silver nanoparticles toxicity. Because of this, as well as their ability to adsorb biomolecules and interact with biological receptors , nanoparticles may be able to reach subcellular

regions, once such particles begin to disintegrate or degrade in situ, larger localized ion concentrations may result. The ability of NPs to form reactive oxygen species, interact with biomolecules such as proteins, enzymes, and DNA, and potentially disrupt their function, further Complicates the situation. Shape, size, surface coating and other factors of NPs contribute these interactions (Reidy *et al.*,2013).

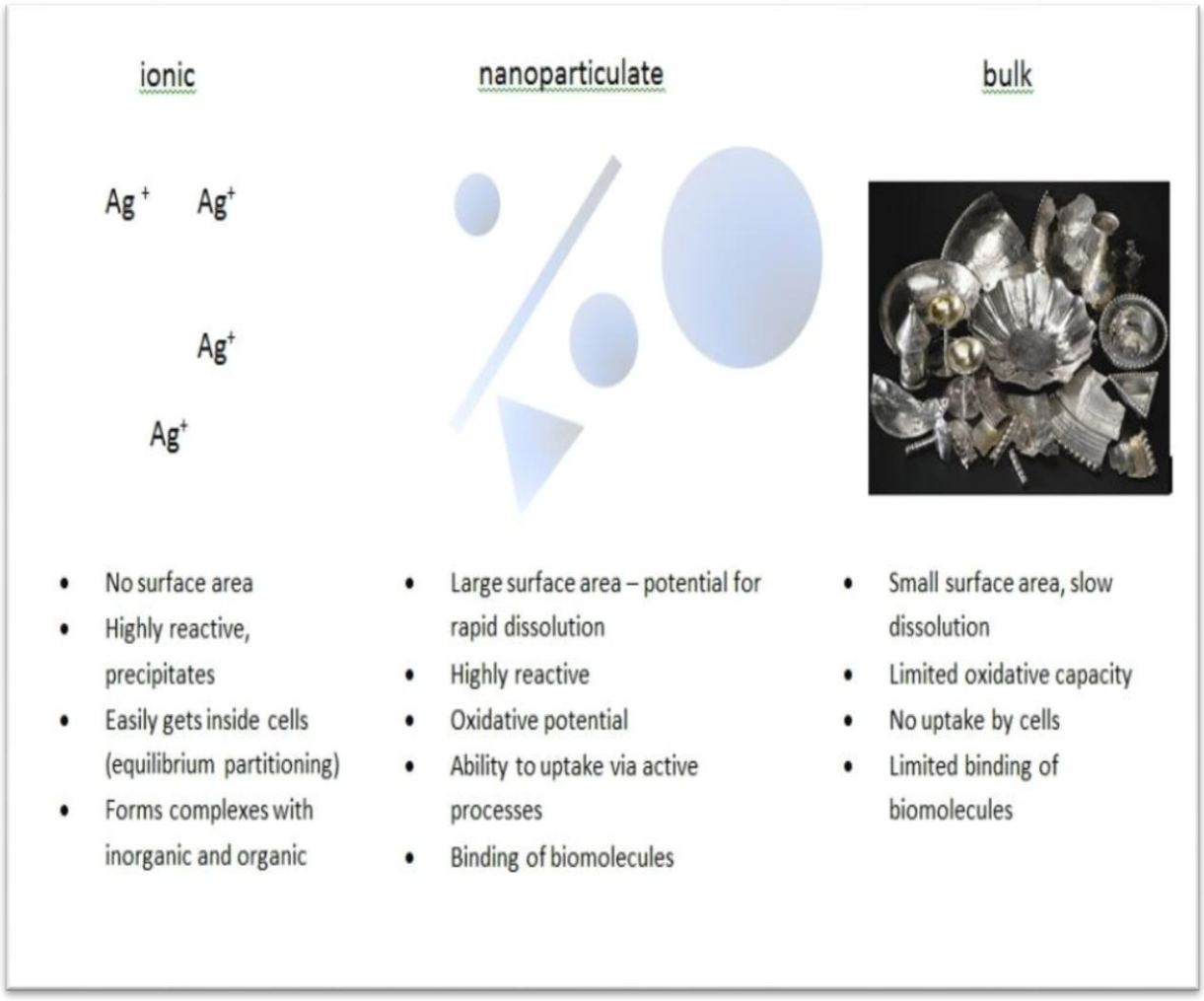


Figure 1.4: Main Differences Between Ionic, Nanoparticle, and Bulk Silver. (Reidy *et al*., 2013)

1.2.6 Silver-Synthesizing Bacteria

The *Pseudomonas stutzeri* AG259 strain, which is discover from a silver mine, provided the first proof of bacteria manufacturing silver nanoparticles.

Some bacteriacan survive and develop in high metal ion concentrations, and this is owing to their resistance to the metal (Hallol,2013). Actinomycetes are a large and diverse group of Gram-positive bacteria that were aerobic and mycelial in nature. They are one of the most important microbial communities in the soil, and they play a crucial ecological function in soil cycles. Actinomycetes are economically significant because they generate antibiotics,vitamins , and enzymes (Devadass *et al.*,2016). Microorganisms make nanoparticles by grabbing target metal ions from their environment and converting them into the element metal using enzymes. Biogenic synthesis of metal NPs from actinomycetes, in particular, can occur intracellularly or extracellularly (Nayaka *et al.*,2020). To synthesize AgNPs, a variety of bacteria have been studied, including *Ureibacillus thermosph aericus* (Juibari *et al.*,2011), *Escherichia coli* (ElShanshoury *et al.*,2011), *S. aureus* (Nanda, & Saravanan 2009) , *P. aeruginosa* (Busi *et al.*,2014), *Bacillus thuringiensis* (Jain *et al.*,2010) , and *Bacillus cereus* (Sunkar and Nachiyar ,2012; Rajora *et al.*,2016). These NPs can be made either intracellularly or extracellularly, Figure (1.5). Bacteria convert Ag^{+} ions to their elemental form Ag^{0}, which is stored outside the cell and resulting in the creation of AgNPs. Extracellularly formed AgNPs come in a variety of forms and sizes , depending on the growth media of bacteria: hexagonal, spherical, triangular, circular, disc, and cuboidal (Oves *et al.*,2013;Otari *et al.*,2014; Elbeshehy *et al.*,2015).

Biogenic reduction of Ag^{+} to Ag^{0} is proteins found on the bacterial cell wall or tiny soluble secretory enzymes such as (nitrate reductase), which aided by membrane proteins. Silver resistant bacteria reduce Ag^{+} to Ag^{0}, Intracellular creation of AgNPs includes silver ion movement within the bacterial cell, which accumulates on the cell wall or in the periplasmic

space, limiting the toxicity of silver salts. Ag^0, which accumulates on the bacterial cell wall, has been reported to account for up to 25% of the mass of the bacterium (Murugan *et al.*, 2014). AgNO3 solution is reduced by *Pseudomonas stutzei* AG2 59, which produces AgNPs with a size of 200 nm as well as a minor amount of mono clinic crystalline-form Ag sulfide (Ag2S), (Klaus *et al.*,1999;Javaid *et al.*,2018).

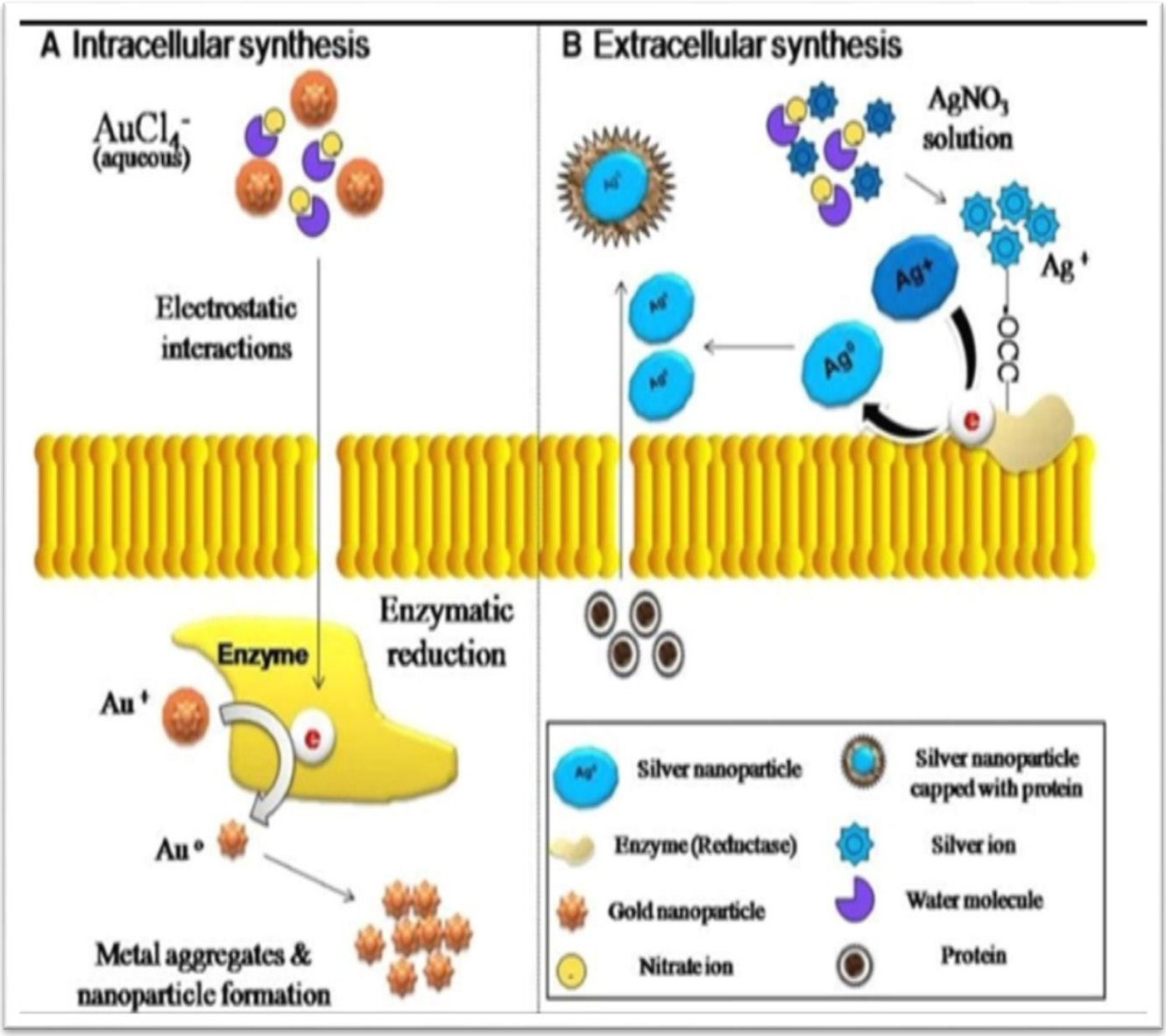


Figure1.5: Intracellular and Extracellular Synthesis of Nanoparticles. (Khandel & Kumar Shahi, 2016).

Below list of bacteria and actinomycetes that synthesis of metal nanoparticle biologically:

Table1.1: Types of Bacteria That Synthesize Metal Nanoparticles. (Manivasagan *et al.*,2015).

Bacteria	Nanoparticles	Size in (nm)
Bacillus megaterium	Silver	46.9
Klebsiella pneumonia	Silver	50
Bacillus licheniformis	Silver	50
Corynebacterium sp	Silver	10-15
Bacillus subtilis	Silver	5 – 60
Geobacter sulfurreducens	Silver	200
Morganella sp	Silver	20±5
Escherichia coil	Silver	1-100
Proteus mirabilis	Silver	10-20
Bacillus sp.	Silver	5-15
Lactic acid becteria	Silver	4-5
Staphylococcus aureus	Silver	1-100
Bacillus cereus	Silver	11.2
Brevibacterum casei	Silver	50
Enterobacter cloacae	Silver	50-100
Pseudomosnas stutzeri	Copper	50-150
Bacillus subtills	Gold	5-25
Pseudomosnas aeruginosa	Gold	15-30
Rhodococcus sp,(Actinomycete)	Gold	5-15
Thermoanaerobacter ethanolicus	Magnetite	36-65
Magnetocpirillum magnetotacticum	Magnetite	50-100
Rhodopseudomonas capsulate	Gold	10-20
Corynebacterium glutamicum	Silver	5-50
Ureibacillus thermosphaericus	Gold	50-70
Pyrobaculum islandicum	Cobalt	-
Desulfovibriodesulfuricans	Lead	50
Nocardia farcinica	Gold	15_20

Table 1.2: Types of Nanoparticles Synthesized From Different Actinomycetes.

Actinomycetes	Type of NPs	References
Streptomyces sp.	Silver	Alani *et al*. 2012
Streptomyces sp.	Silver	JAR1 Chauhun *et al*. 2013
Thermoactinomyces sp.	Silver	Deepa *et al.* 2013
Nocardiopsis sp. MBRC-1	Silver	Manivasagan *et al*. 2013
Actinomycetes	Silver	Narasimha *et al*. 2013
Rhodococcus sp.	Silver	Otari *et al*. 2012
Streptomyces albidoflavus CNP10	Silver	Prakasham *et al*. 2012
Streptomyces hygroscopicus BDUS 49	Silver	Sadhasivam *et al.* 2010
*Streptomyces sp.*VITPK1	Silver	Sanjenbam *et al*. 2014
Streptomyces rochei	Silver	Selvakumar *et al.* 2012
Streptomyces sp. BDUKAS10	Silver	Sivalingham *et al*. 2012
Streptomyces sp. VITBT7	Silver	Subashini and Kannabiran 2013
Streptomyces sp. I, Streptomyces sp. II,	Silver	Sukanya *et al*. 2013
Rhodococcus sp.	Gold	Ahmad *et al*. 2003b; 2003c
Streptomyces aureofaciens MTCC356	Silver	Sundarmoorthi *et al*. 2011

Actinomycetes sp.	Silver	Sunitha *et al.* 2014
Streptomyces glaucus 71MD	Silver	Tsibakhashvili *et al*. 2011
Streptomyces aureofaciens MTCC356	Silver	Vengadesh Prabu *et al*. 2011
Streptomyces sp. JF741876	Silver	Vidyasagar *et al*. 2012
Streptomyces sp. ERI-3	Silver	Zonooz and Salouti 2011
Streptomyces sp. LK3	Silver	Karthik *et al*. 2014
Streptomycetes viridogens HM10	Gold	Balagurunathan *et al*. 2011
Thermoactinomycete spp. 44Th	Gold	Kalabegishvili *et al*. 2013
Nocardia farcinica	Gold	Oza *et al*. 2012
Thermomonospora sp.	Gold	Sastry *et al*. 2003
Streptomyces hygroscopicus	Gold	Waghmare *et al*. 2014
Streptomyces sp.	Zinc	Rajamanickam *et al*. 2012
Streptomyces sp.	Zinc/copper	Usha *et al*. 2010
Streptomyces sp. HBUM171191	Zinc/manganese	Waghmare *et al*. 2011

1.2.7 Identification of the Isolates

1.2.7.1 Genotypic Identification

Woese and colleagues demonstrated that phylogenetic relationships of bacteria, and all life forms, may be identified by comparing a stable component of the genetic code. The gene that code for the 5S, 16S (also known as the small subunit), and23S rRNA, as well as the ga ps between these genes, are candidate for this genomic region in bacteria. The 16S rRNA gene can be compared not just among bacteria, but also with archeobacteria's16S rRNAgene and eukaryotes'18S rRNA gene.The 16SrRNA gene sequence is approximately 1,550bp long , with both variable and conserve sections.

The gene is large enough to produce differentiating and statistically significant measures, with enough interspecific variations in the 16SrRNA gene. The conserved portions at the start of the gene and at either the 540bp region or the end of the whole sequence (about the 1,550bp region) are usually complementary to universal primers. The comparative taxonomy is based on the sequence of the variable area in between. Although sequences of 500 and 1,500bp are commonly used to compare and sequence, sequences in databases can be of any length. Consider if sequencing the entire 1,500bp length is necessary or whether the ty pically reported shorter sequences can give comparable information.To identify between cert ain taxa or strains, it is sometimes required to sequence the full 1,500-bp area.

When characterizing a novel species, sequencing the whole 1,500 bp sequence was desirable and typically essential. Because the 16S rRNA gene was founded in all bacteria, associations can be assessed across species. Generally The comparison of 16S rRNA gene sequences allows for genus level distinction across all major phyla of bacteria , as well as classification of strains at various levels, including what we now call species and subspecies (Clarridge , 2004).

1.2.8 Physical Characterization of NPs

1.2.8.1 UV-Vis Spectroscopy

Each material's molecules absorb visible light at a specific wavelength (WL), which was determined by the chemical nature of the material, it was highly conjugated chemical structure, and the presence of transition metal ions. UV-Vis spectroscopy examines molecules under going electronic changes using light in the visible and near ultraviolet (NUV) ranges (Forough & Farhadi,2010).

1.2.8.2Transmission Electron Microscopy (TEM)

Transmission electron microscopy (TEM) is a technique that involves sending an electron beam through an ultrathin object and interacting with it as it passes through. This technique uses energetic electrons to provide crystallographic, morphologic, and compositional data on samples. It's frequently used in biology and nanotechnology, and it's very good in obser ving metal nanoparticles (Cooper *et al*.,2016).

1.2.8.3 Scanning Electron Microscopy (SEM)

SEM gives topographical and elemental information on NPs at relevant magnifications and with a virtually infinite depth of field .SEM can be used to determine the elemental NPs, composition, grain size, surface roughness, porosity, size distributions, homogeneity, inter metallic distribution, and diffusion (Palmqvist, 2017).

1.2.8.4 X-ray Diffraction (XRD)

Physical properties, crystallographic structure, and chemical composition of materials were studied using X-ray diffraction techniques, which were widely used and very valuable non-destructive characterization tools.

It can also be utilized to characterize various crystalline phasing structural properties, such as phase composition, grain size, strain and defect structure, (Sharma *et al*., 2012).

1.2.8.5 Energy-Dispersive Spectroscopy (EDX)

Surface analysis and elemental characterisation of a sample was investigated using energy dispersive spectroscopy. The examination of the released Xrays of various energy from the the sample when an electron beam contacts its constituents the basic premise. The amount and composition of metal NPs may be easily determined by looking at the sample's surface (Smuleac *et al*., 2013).

1.2.8.6 Atomic Force Microscopy (AFM)

AFM is a sophisticate nano scope technique used to characterize nanoparticles and conduct three-dimensional surface investigations of nanomaterials. Because AFM pictures maybe acquired in an aqueous media, it is a useful tool for studying the behavior of NPs in a biological setting (Bhosale *et al*., 2014).

1.2.9 Biological Characterization

1.2.9.1 Antimicrobial Activity of AgNPs

It's known to have bactericidal and inhibiting properties. Pathogenic bacteria have developed resistance to antimicrobial drugs in recent years, and this has become a major public health issue. *Pseudomonas spp., Acinetobacter baumannii, Klebsiella pneumoniae,S. aureus*, and *Streptococcus agalactia* have always been a global problem in public health, resulting in millions of deaths each year (Giovanna *et al.,* 2016). Multidrug resistant bacteria can be treated using a variety of approaches, including the discovery of novel antimicrobial medicines. Nano bio-technology, which uses NPs with

unique physicochemical properties, has recently been developed as a new technology for combating multidrug-resistant (MDR) pathogens (Singh *et al.,* 2018).

The synthesis of silver metallic bio-nanoparticles using aqueous Ag^+ ion reduction with Staphylococcus aureus culture supernatants.

The aqueous component's bio reduction of Ag^+ ions was monitored,(Nanda, and Saravanan,2009).Biogenic silver nanoparticles have antibacterial properties. In terms of the antimicrobial efficacy of the prepared silver nanoparticles, the lethal mechanism against the tested pathogenic microbes could include the release of silver ions from silver nanoparticles and the consistency of crystalline carbohydrates, proteins, lipids, and nucleic acids, assembled on the microbial walls, with silver nanoparticles steady forming cavity and piercing to the cytoplasm inside the plasma membrane.

AgNPs may connect to the outside of the plasma membrane, disrupting the cell's permeable process and respiratory activities, or by interfering with components of the microbial electron transport system, according to other research (Sharma *et al.*, 2009).

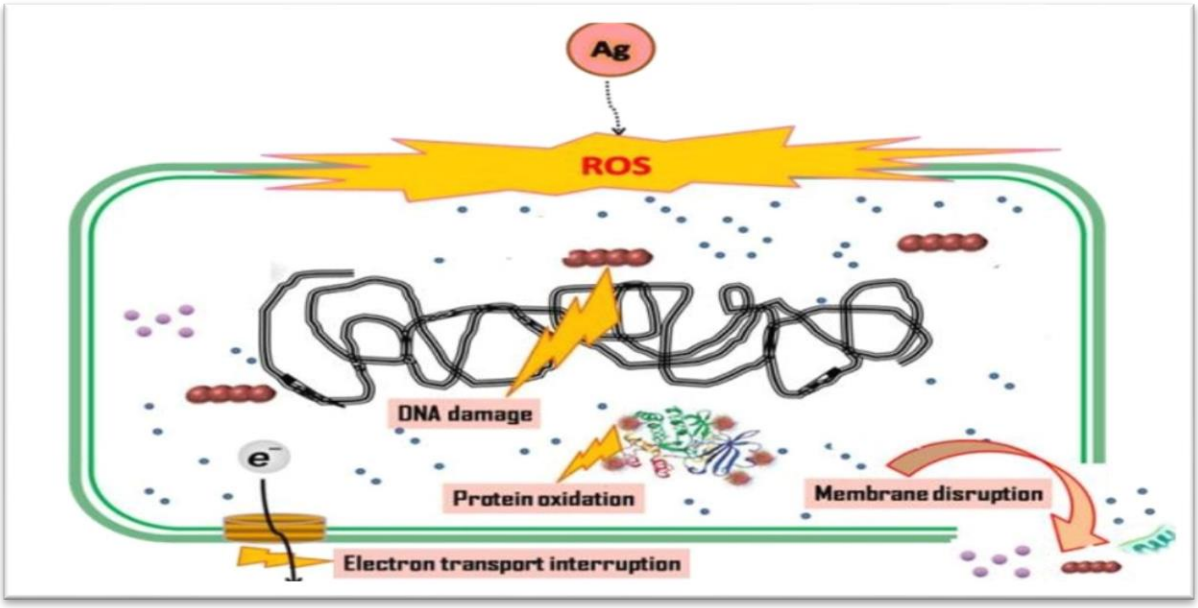


Figure 1.6: Mechanism of Antibacterial Activity by Bio-AgNPs (Dayma *et al.*, 2019).

1.2.9.2 Antitumor Activity of Biosynthesized AgNPs

Chemotherapy was one of the most common treatments for cancer, and a large number of antitumor compounds are found in nature as a whole or as derivatives, mostly formed and produced by microorganisms, particularly actinomycetes,which produce a large number of natural products with various biological, bioactive properties, and antitumor properties (Olano *et al*., 2009).

These anticancer chemicals were came from a variety of structural classes and they work by inducing apoptosis through one of the suitable mechanisms, figure(1.7).

Topoisomerase I or II inhibition causes such DNA cleavage. Inhibition of important enzyme affects signal transduction, such as proteases, mitochondrial permeabilization, and cellular metabolism, as well as tumor induced angiogenesis in some situations (Olano *et al.*,2009).

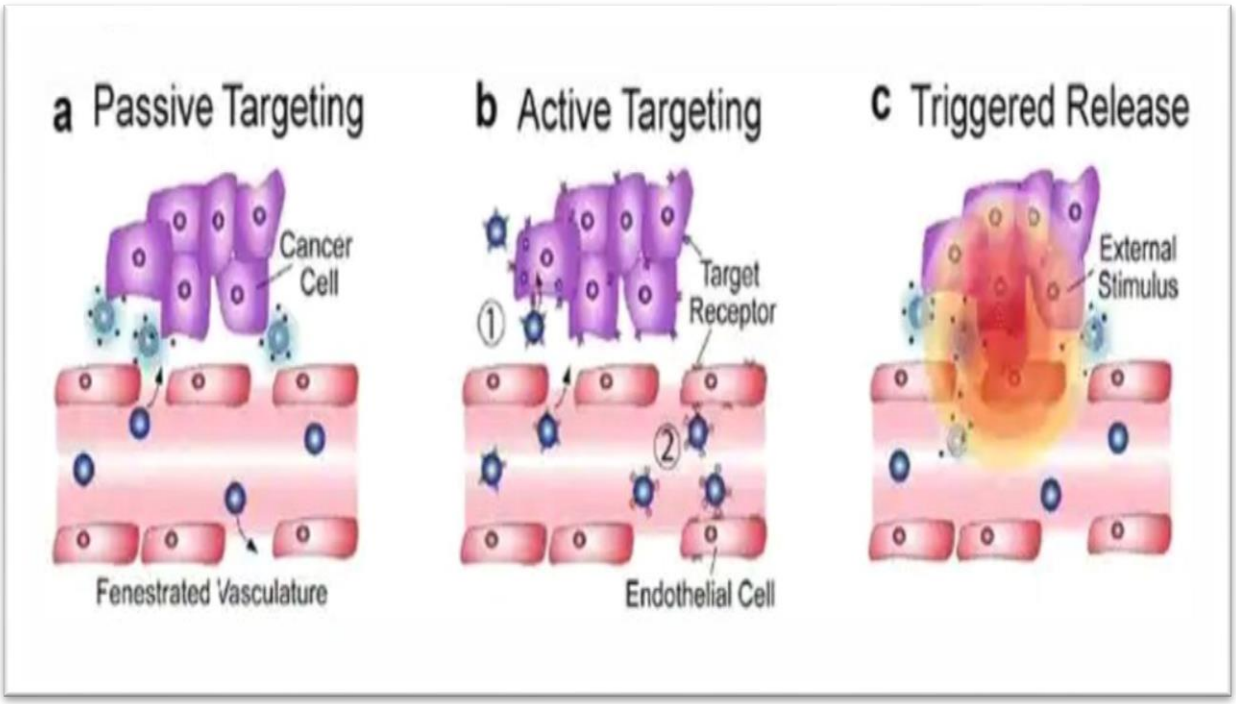


Figure 1.7: Type of Targeting for Nanoparticle Delivery to Tumor Tissue. (Tran,2017)

Natural products are the most consistently successful sources of medication safe, with just 42% of the 175 anticancer medicines identified between the 1940 and2006 being natural products or compounds derived from them. Many anticancer medications found, on the other hand, were natural compounds isolated and purified from microbes (particularly actinomycetes) or plants thriving in ecological niches.(Gullo *et al.*, 2006; Newman and Cragg 2007; Amin,2016).

1.2.10 Breast Cancer

Breast cancer was the most frequent cancer in women all over the world. It was the greatest cause of cancer death in females globally, accounting for 15% of all cancer fatalities in(Jin, 2020) in women. According to the Iraqi cancer registry in 2004, it was the most frequent type of female malignancy, accounting for around one_third of all recorded were cancers in female (Ismaeel, 2013; Mohammed,2011). About 5-10% of breast cancer is inherited (Al Hannan *et al*; 2019). BRCA1 and BRCA2 gene mutations increase the risk of breast and ovarian cancer, however the typical magnitude of these hazards was unknown and may vary depending on the environment. Estimates based on multiple case families may be enriched for higher risk mutations and or other familial risk factors , but risk estimates based on patients without a family history have been sloppy. The pedigree data from 22 studies involving 8,139 index case patients with female (86%) or male (2%) breast cancer or epithelial ovarian cancer (12%), (Antoniou *et al*.,2003).

CHAPTER TWO

MATERIALS & METHODS

2. Materials and Methods

2.1 Materials

2.1.1 Equipment and Apparatus

In this study, the following apparatus and equipment were used:

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

No.	Equipment and apparatus	Company / origin
1	AFM	Buker/Germany
2	Autoclave	Hirayama/Japan
3	Beakers	Iso Lab/Germany
4	Burner	Indiamart/India
5	Cell culture plates	Thermo Fisher Scientific/USA
6	Cooling Centrifuge	Eppendroff/Germany
7	Cotton swabs	John Bolten/england
8	CO2 incubator	CypressDiagnostics/Belgium
9	Disposable Petri dishes	Al-Hanicompany/Lebenon
10	DNA Bacteria Kit	Geneaid, Taiwan
11	EDX	Broker/Germany
12	Electrophoresis apparatus	Bioneer/Korea
13	Eppendrof tubes	BDH/UK
14	flasks	Iso Lab/Germany
15	Gel Documentation system	Biometra/Germany
16	Glass spreader	John Bolten/England
17	Incubator	Human Lap/Korea
18	DNA Ladder	Intronbio/ korea
19	Laminar flow hood	K&K Scientific Supplier/Korea
20	Light Microscope	Olymps/Japan
21	Maxime^{TM} PCR PreMix	Intronbio/ korea
22	Microwave	Shonic/China
23	Micropipettes	DraonMED/China
24	Microtiter reader	Thermo Fisher Scientific/USA
25	Oven	Memmert/Germany

26	Parafilm	BEMIS/USA
27	Primer	Macrogen/Korea
28	quartz cuvette	Lab.Tech /Korean
29	Refrigerator	Vistal/Poland
30	Shaking incubator	Lab.Tech /Korean
31	Sensitive Balance	Sartorius/Germany
32	SEM	Buker/Germany
33	Slides and covers	Superestar/India
34	Sterile tiny plastic bags	Superstar/India
35	Test Tubes	AFCO-Dispo/Jordan
36	TEM	Broker/Germany
37	Tex mixture Vor.	Medilab/Korea
38	Thermal cycler apparatus	Bioneer/Korea
39	Tips	SterellinLtd/UK
40	trowel	SML/KSA
41	UV spectrophotometer	Shimadzu/ India
42	UV. Transilluminator	Elettrofor/Italy
43	Vitek2 Compact system	Biomerieux/France
44	Vitek2 GN Kit for Gram negative	Biomerieux/France
45	Vitek2 GP Kit for Gram positive	Biomerieux/France
46	Vortex	CYAN/Belgium
47	Water path	Memmert/Germany
48	Whatman No.1 Filter paper	Sigma/USA
49	Wire Loop	John Bolten/England
50	XRD	Broker/Germany

2.1.2 Media and Chemicals

2.1.2.1 Media that Prepared in Lab

Table 2.2: Preparation of Special Culture Media for Isolating Bacteria.

No.	Culture Media	Abbreviation	Composition	Amount
1.	Starch-Casein-Nitrite Agar (Bernard, 2007)	SCN Agar in 1000ml D.W	1. Starch	1.0 g
			2. Casein	0.4 g
			3. Potassium Nitrate	0.5 g
			4. Potassium Monohydrogen Phosphate	0.2 g

			5. Magnesium Phosphate	0.1 g
			6. Calcium Carbonate	0.1 g
			7. Agar	15 g
2.	Yeast Extract Glucose Agar (Bernard, 2007)	YEG Agar in 1000ml D.W	1.Glucose	10 g
			2.Yeast Extract	1.0 g
			3. Potassium Nitrate	1.0 g
			4. Potassium Monohydrogen Phosphate	0.1 g
			5.Agar	15 g
3.	Malt extract Glucose Yeast Extract Peptone Broth(Dayma *et al*.,2019)	MGYP broth in 1000ml D.W	1.Malt extract	0.3 g
			2.Yeast extract	0.3 g
			3.Glucose	1.0 g
			4.Peptone	0.5 g

2.1.2.2 Culture Media (Ready to Use)

Table 2.3: Culture Media

No.	Culture Media	Company/Origin	Amount
1.	Nutrient Agar	Himedia/India	28 g in 1000ml D.W
2.	Mueller Hinton Agar	Michigan/USA	38 g in 1000ml D.W
3.	Blood Agar Base	Himedia/India	23 g in 1000ml D.W
4.	MacConkey Agar	Himedia/India	111g in 1000ml D.W
5.	Chocolate Agar	Himedia/India	50 g in 1000ml D.W

All media sterilized in 121^{o}C autoclave for 15 min.

2.1.2.3 Chemicals

Table 2.4: All Chemicals Used in the Study with the Name of the Company Manufacturer and Country of Origin

No.	Chemicals	Company/origin
1.	AgNO3 Silver Nitrate	Roth/Germany
2.	Cycloheximide	Himedia/India
3	Glycerol	Himedia/India
4.	Deionized Distilled Water	Capricon/USA
5.	Trypsin/EDTA	Capricorn/USA
6.	DMSO	Santa Cruz/USA
7.	RPMI 1640	Gibco/USA
8.	MTT Stain	Sigma/USA
9.	Fetal Bovine Serum	Gibco/USA
10	Gentamicin Disks	Liofilchem s.r.i./Italy

2.2 Methods

2.2.1 Soil Samples Collecting

Forty,Soil samples were gathered from several locations, including sugar cane fields and gardens, from(24November 2020 to 24May 2021). Samples were taken from a depth of (11 mm). The soil was dug with a trowel, the samples were gathered in sterile tiny plastic bags

Figure 2.1: a, b, Soil Sample Collecting

that were clearly labeled with the date and place of collection. As shown in figure (2.1).

2.2.1.1 Soil Samples Drying

In this study Some of the soil samples were dried for roughly three hours in a hot air oven at 60^{o}C in biology laboratory/college of science / Misan university. This was done to reduce the number of bacteria other than actinomycetes in the soil. Actinomycetes were produced spores in the soil that germinate and develop in the media, this is one of many methods for isolating actinomycetes from soil samples selectively, then the samples were transferred to tubes, and keep at 4^{o}C **(**Pharm,2008).

2.2.1.2 Isolation Media

In this study, actinomycetes were isolated using a starch-casein-nitrate agar media as an isolation medium, while other bacteria isolates using Nutrient agar, SCN agar medium was made, the components were placed in the flask, which then plugged with cotton and the pH was check and adjust to (7.2) before being sterilized in121°C autoclave for fifteen

minutes. The medium is then allow to cool to roughly 50°C then adding Cycloheximide (1ml) at concentration of (100ug/ml) before being poured over the plates **(**Pharm,2008).

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 the test tube 1 and mixed well. One ml of soil solution from tube 1 was transferred to tube 2 using a I ml micropipette and mixed well. This serial dilution was done up to tube 4. 1ml solution was discarded from the tube 4. Thus the concentrations of 10^{-1},10^{-2},$10^{-3}$and 10^{-4} g/ml were obtained **(**Pharm,2008) with minor modification.

2.2.1.4 Dilution Plating

A total of four Petri dishes containing isolation media were labeled 1,2,3, and 4 to each dilution's test. Starting with tube4, transfer 0.1ml of the solution to the Petri plate that had been labeled. Using a sterilize glass spreader, each soil solution distributed on the agar medium immediately after transfer. The plates were then incubate for five days at 30°C **(**Pharm ,2008).

2.2.1.5 Pure Culture Isolation

The media was made according to the instructions After that, the medium (Yeast Extract Glucose agar) is heat in order to dissolve the ingredients. After Obtaining the clear solution, it was autoclaved for 15 minutes at 121°C. The medium is then allow to cool to roughly 50°C and(1ml) of cycloheximide (100µg/ml) was added before poured over the plates, then the medium poured onto the plates, **(**Pharm,2008).

2.2.1.6 Transfer of Isolated Colonies to Transfer Medium and Incubation

The well isolated colonies were carefully scrap and streaked on the transfer media and N. agar using a flamed loop. On one plate of transfer material, one colony was transferring. The plates were then incubated for 7 days at 30°C. After the incubation period The plates were tested for any contamination. The plate that was not contaminated, consider as pure culture. Bacteria was isolated as single colonies on YEGA and Nutrient agar from each sample. The colonies were spreaded onto a microscopic slide, heat fixed, and gram stain (1min crystal violate. rinse with water, Gram's iodine 1minute, rinse with water, then decolorize with 95% ethyl alcohol, rinse with water, safranin-counter-stain-for1min),for microscopic identification (MacFaddin,2000). Also we used cotton blue stain for dyeing actinomycetes **(**Pharm, 2008).

2.2.1.7 Morphological Identification

The plates were examined after the incubation period, colonies were examined to see the morphological feature, if they tiny, opaque, compact, pigmented or dull (Bernard,2007). Under a light microscope, the colonies were examined for colonial morphology and to differentiate other bacteria from actinomycetes. The growth of fungi on the plates was excluded, because of Cycloheximide that was added to the media.

2.2.1.8 Pure Cultures Maintenance and Preservation

It was one of the most commonly utilized preservation techniques by microbiologists (Demain and Davies,1999). This technique was employed in this project for stock culture maintenance and agar slant preparation. preparation of YEGA medium (transfer medium) and Nutrient agar, then transferred to test tubes in volumes of about 5ml, the tubes were then kept on a slant tray. Pure cultures were transferred to test tube slants and cultured at

30°C for 5days. The tubes were then checked, and if the organism's growth was founded to be satisfactorily, they were covered with Parafilm and stored at 4°C in the refrigerated for future use ((Harley and Prescott, 2002; Hassan,2018).

2.2.2 Molecular Identification of the Isolates

2.2.2.1 16S rRNA Gene Sequencing

Genomic DNA was isolated from isolates using universal primer for all samples. As shown in table (2.5).

Table 2.5: Sequence of Primer Kite that Use in the Experiment

Primer	Sequence (5´- 3´)	Length	Amplicon Size	Reference
27F	´ AGAGTTTGATCCTGGCTCAG	20bp	1500bp	Miyoshi, *et al*, 2005
1492R	GGTTACCTTGTTACGACTT	19bp		

2.2.2.2 DNA Extraction

Genomic DNA was extracted from isolates by (Presto ' Mini g DNA Bacteria Kit Quick Protocol TM, Catalogue Number DO GBB004, GBB100/101, GBB300 /301) methods.

PCR reaction mixture with final volume 25µl consisted of 2µl for each 27F and1492R primers (10 picomole), 9µl De-ionized water, and 7µl of the DNA of the isolate, were added into the Maxime TM PCR Premix i-Taq (Kim *et al*,2007).

2.2.2.3 PCR Conditions

The extracted DNA was amplified by polymerase chain reaction (PCR) technique, the following PCR conditions were:

An initial denaturation at 94°C for 1min. followed by 30 cycles of denaturation at 94°C for 1min. annealing at 58°C for 30 sec. and extension at 72°C for 1 min, with a final

extension at 72°C for 7 min. The PCR product that amplified be then Electrophoresis on Agarose Gels (Pharm,2008).

2.2.2.4 Agarose Gels Electrophoresis

The agarose used in electrophoresis was prepared by dissolving 1g or more of agarose with one hundred ml TBE 1X buffer. The solution was heated by microwave until boiling point where all the gel particles were dissolved, left to cool at 50°C.Three µl of Ethidium bromide 0.5µg/ml was added to agarose and poured in preparing tray with fixing of a comb the agarose was left to solidify at room temperature for 30 minute. The comb was removed after hardening of agarose leaving well The first well was loaded with (5µl) of DNA ladder and each well was loaded with (5µl) of PCR product. After an 80min. of electrophoreses at 80 volts, the gel was viewed and photographed using a gel documentation system, (Sambrook and Russell,2006; Mishra*et al*,2010).

2.2.2.5 Identification of the Isolation on GenBank

The product of PCR was sent to the lab of biotechnology for sequencing at the national instrumentation center for environmental management (NICEM) online using DNA Sequencer 3730XL, Applied Biosystem. Homology search was conducted using Basic Local Alignment Search Tool (BLAST) software which is available at the National Center of Biotechnology Information (NCBI) web site online at (http:/www.ncbi .nlm .nih.gov), (Hassan,2018).

2.2.3 Biosynthesized of Silver Nanoparticles in Laboratory

2.2.3.1 Biosynthesis of Silver Nanoparticles from Isolated Bacteria

Bacterial strains that have been isolate, purified, and identified were cultured in MGYP broth (pH 7) at 26°C for 7 days with constant shaking on a shaker incubator (150rpm).

Through Whatman filter paper (No.1) biomass was filtered after incubation to separated it from the supernatant then the medium was centrifuged to obtain supernatant, two solutions were made to make AgNPs:

the first is made by mixing (v/v)200 ml of supernatant with 200 ml of AgNO3 (2mM). As a control, a second reaction mixture is made without AgNO3. The interaction took place at (pH7). The solutions were kept on a rotary shaker (150rpm) at 26°C for 7days in the dark (to prevent photochemical reversal during the experiment).The appearance of a color change in the culture solution was the first indicated for the development of Bio-AgNPs (Dayma *et al.*,2019).

The extracellular synthesis of silver nanoparticles done by using the supernatant. Bacteria biomass is collect for intracellular synthesis at the same time, in a 250ml flask, roughly 2 g of wet biomass was re-suspended in100ml of 2Mm aqueous AgNO3 solution(Das *et al.*, 2014).

The mixture was separated for 20 minutes at 8000 rpm in centrifuge, the supernatant replace with DDW centrifuge four times, and the pellets were dried at 45°C for 30 hours. For addition testing, the powder is retain in vials, (Aldujaili *et al.*,2020) with minor modification.

2.2.3.2 Characterization of Biogenic AgNPs

Physical and biological characterization were the most common methods for determining the properties of nanoparticles.

2.2.3.2.1 Physical Characterization of Biogenic AgNPs

Two of the main factors that have been studied in the characterization of nanomaterials were size and shape. We can also measure volume distribution, degree of aggregation, surface charge, surface area and to some extent evaluate surface chemistry (size, volume

distribution, and organic bonds on the surface of the particles may influence other properties) and their potential applications. In addition, their crystal structure and chemical composition are thoroughly investigated as a first step after NPs synthesis (Verma and Maheshwari,2018).

2.2.3.2.1.1 UV- Spectroscopy

A UV–visible spectral scan at 300-800nm was used to ensure the formation of silver NPs. Both treated and untreated solutions centrifuged for 5min. at 2000 rpm. The untreated supernatant set as reference control while treated supernatants used to monitor their UV-Visible absorbance Spectra between 300-800 nm wave length.

Silver nanoparticles have a broad UV visible spectrum indicating the presence of silver nanoparticles (Soltaninezhad *et al*, 2015).

2.2.3.2.1.2 TEM Examination

The formation shape and size of the generated silver nanoparticles were determined by Transmission Electron Microscopy examination, according to micrographs, (Williams and Carter, 2009).

2.2.3.2.1.3 XRD Analysis of AgNPs

X-ray diffraction (XRD) was one of the most widely used techniques for characterizing AgNPs. XRD usually provides information regarding crystal structure, phase nature, lattice dimensions, and crystal sizes. The latter is estimated using Scherer's equation using the mid-high atom widening of the diffraction angle in the XRD measurement of a given sample. Particle composition can be determined by comparing the position and intensity of the peaks with reference patterns.

2.2.3.2.1.4 Scanning Electron Microscopy (SEM)

SEM was using to examine the AgNPs in the sample. Thin films of the sample were made on carbon-coated copper grids by dropping an amount of the filtrate on the given sample. Grid and blotting away the excess solution using blotting paper, then allowing the films to dry over night at room temperature under sterilized conditions. The silver nanoparticle was imaged using a scanning electron microscope equipped with EDX attachment (Fissan *et al* 2014).

2.2.3.2.1.5 Analysis Using an Atomic Force Microscope (AFM)

Size dispersion and aggregation of nanomaterials were investigated using an atomic force microscope (AFM). Atomic force microscopy was used to examine sufficient amount of biogenic AgNPs (Vinelli *et al*.,2008).

TEM, XRD, SEM, EDX and AFM were examination at BPC-Analysis center in Baghdad to determinate the physical properties of biogenic nanoparticles synthesized laboratory.

2.2.3.2.2 Biological Characterization of Biogenic AgNPs

Methods used for characterization of biogenic AgNPs in this study were Antibacterial and antitumor activity (Tagliette *et al*,2012).

2.2.3.2.2.1 Antibacterial Properties of Synthesized AgNPs

The antibacterial activity of the AgNPs produced was examined using the method (agar disc diffusion) against several pathogenic bacteria isolate from clinical specimens, that identification were carried out by using -VITEK2 (Thapa,2017; Abbaszadegan *et al.*, 2015).

2.2.3.2.2.1.1 Isolation of Pathogenic Bacteria from Hospital

The specimens were isolated from patients in AL Sadr hospital in Misan, Iraq, thirty- one specimens were collected from ear, urine, wounds, burns, blood, and discharge. The swap was used to culture the specimens on blood agar and MacConkey agar at 37 ^{o}C for one day incubation in the hospital.

2.2.3.2.2.1.2 Identification of Pathogenic Bacteria Using VITEK2

The Vitek - 2 device was been used to diagnosis of bacteria isolates from culture media sterile swabs was utilized to transport one to two colony of pure culture then suspended bacteria in (0.3) ml of pasteurized brine in clear Polystyrene test tube turbidity is adjust according to mcfarland turbidly rang from in gram negative and gram positive (0.5-0.63) then the cassette bear with 10 Card and suspense , tube and bar code to input data , then loading in to automatri transport system (Silverstein,*et al*., 2000).

2.2.3.2.2.1.3 Preparation of AgNPs Discs

1. In test tube 10µg was put of each synthesized AgNPs .
2. 1ml of DMSO was added to the test tube.
3. A suspension was made by Shaking the test tub very well.
4. A sterile filter paper (6 mm) discs were left in the suspension and shake well.
5. The sterile paper disc was Left to dry (Gould *et al.*,1952).

2.2.3.2.2.1.4 Disc Diffusion Method

Pathogenic bacteria were subculture on transfer media nutrient agar and cultured at 37 ^{o}C for one day. Then, using sterile swabs, standardized suspensions of each teste bacteria (1.5×10^{8} cell/ ml) by McFarland standard 0.5N, (CLSI) swabbed separately onto sterile Muller Hinton agar plates, waited 2-3minutes,then we put AgNPs discs. The discs were

gently press down to guarantee contact. The plates were incubated right away or within 30minutes. Petri dishes were evaluated for the inhibitory zone measure in millimeters after incubation for 24 hours at37°C,(Abdul-hassan,2016). As a control gentamicin disc (10 µg) was used to compare with AgNPs disks.

2.2.3.2.2.2 Antitumor Study of the Synthesized AgNPs in Vitro

Mcf7 (breast cancer cells) were obtained from the IRAQ Biotech Cell Bank Unit in Basra and cultured in RPMI 1640 with 10% Fetal bovine serum, 100 units/ml penicillin , and 100 g/mL streptomycin. Cells were passaged twice a week with Trypsin-EDTA, reseeded at 50% confluence, and incubated at 37 °C with 5% CO2 (Al-Ali *et al.* ,2022).

2.2.3.2.2.2.1 Combination Cytotoxicity Assays

The Methyl Tetra Zolium (MTT) cell viability assay performed on 96 well plates to detect the cytotoxic effect. The mcf7 cell line was planted at 1×10^4 cells per well. Cells were treated with the tested substance at a final concentration of 1000ug/ml after 24hours hours or when a confluent monolayer was attained. After 72 hours of treatment , cell viability was determined by removing the media adding 2 mg/ml MTT solution and incubating the cells for 2 hours at 37°C. Following removal of the MTT solution , the crystals in the wells were solubilized by adding DMSO (Dimethyl Sulphoxide) and incubating at37°Cfor 15 min. with shaking (Sangour *et al*.,2021)with some modification. The absorbency was measured using a microplate reader at 620 nm (test wavelength), and the assay was done three times . The following equation was used to compute the rate of cell growth inhibition:

cytotoxicity = B/A*100,whereB is the mean optical density of treated wells A is the mean optical density of untreated wells, (Freshney,2010).

CHAPTER THREE

RESULTS & DISCUSSIONS

3.Results and Discussions

3.1 Isolation of Bacteria from Soil

As shown in figure (3.1) The results were gathered from all soil sources. Selected (12) colonies were characterized based on their differences in colony morphology and variations in colors (white , black, gray, purple, pink, and yellow), and the texture range from powdery to gelatinous this results agreed with (Amin,2016), there were three types of actinomycetes bacteria from the twelve bacteria those isolates from soil they were: *Streptomyces venezuelae*, *Microbacterium lacticum* and *Microbacterium paraoxydans* which isolated on YEGA, the Earthy odor of the three isolates were present, which consider as distinctive smell of actinomycetes. The rest of the isolates were bacteria isolated on NA (table 3.1).

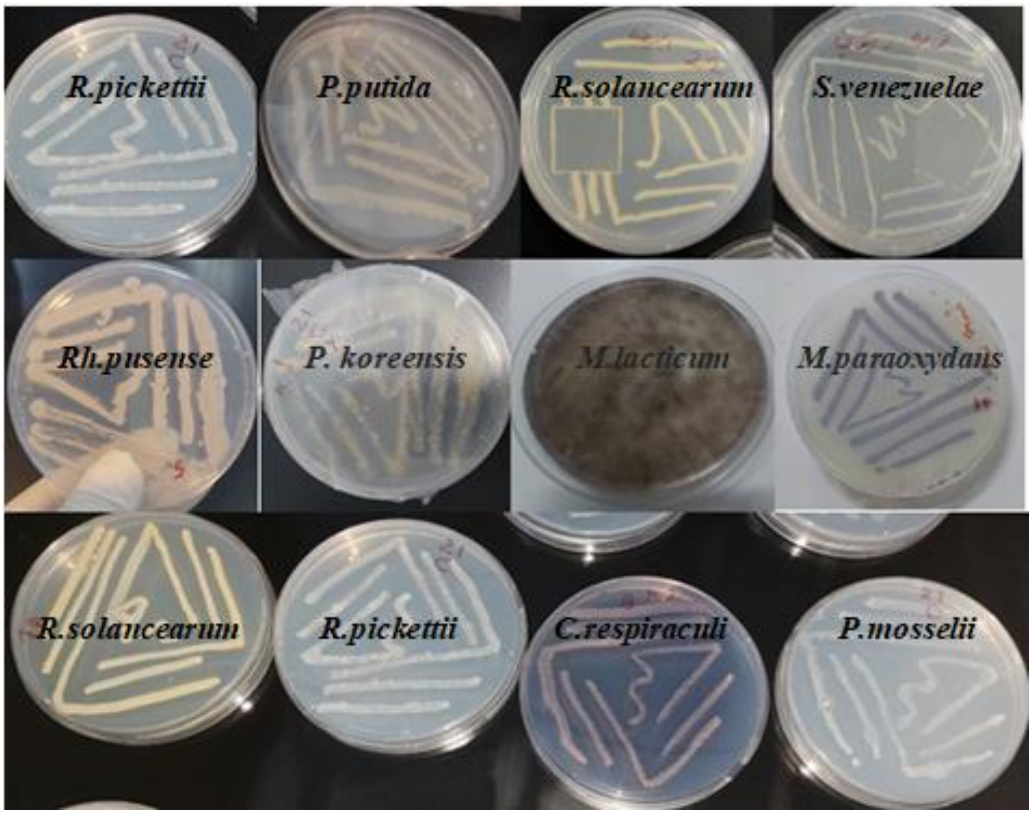


Figure 3.1: Pure Culture of Isolated Bacteria on YEGA and Nutrient agar.

Table3.1 Different Feature of Isolated Bacteria

Isolated Bacteria	Color	Growth	Earthy odor	Texture	Strain
Streptomyces venezuelae	White	v.good	present	gelatinous	JmKmYa12
Microbacterium lacticum	Black	v.good	present	Branched threads	STM54
Microbacterium paraoxydans	gray	v.good	present	gelatinous	shahooda
Pseudomonas mosselii	White	v.good	absence	powder	XG1-1-2
Pseudomonas koreensis	Transparent	v.good	absence	powder	Allawy
Cupriavidus respiraculi	purple	v.good	absence	gelatinous	Halema
Ralstonia solanacearum	Yellow	v.good	absence	gelatinous	Thuraya 9
Ralstonia pickettii	White	v.good	absence	gelatinous	Dewan7
Rhizobium pusense	pink	v.good	absence	powder	WTB70
Pseudomonas putida	Transparent	v.good	absence	gelatinous	LW
Ralstonia pickettii	White	v.good	absence	gelatinous	CHP10
Ralstonia solanacearum	Yellow	v.good	absence	gelatinous	Mehbas

3.2 Microscopic Observation

Light microscope was used to differentiated between actinomycetes and other bacteria. On light microscope the thread on some isolation were seen, by using cotton blue stain that means we obtain actinomycetes isolates, figure (3.2:a, b) other isolates have no thread on light microscope *P.mosselii* Bacteria staining by gram stain, figure (3.2:

c,d)o.

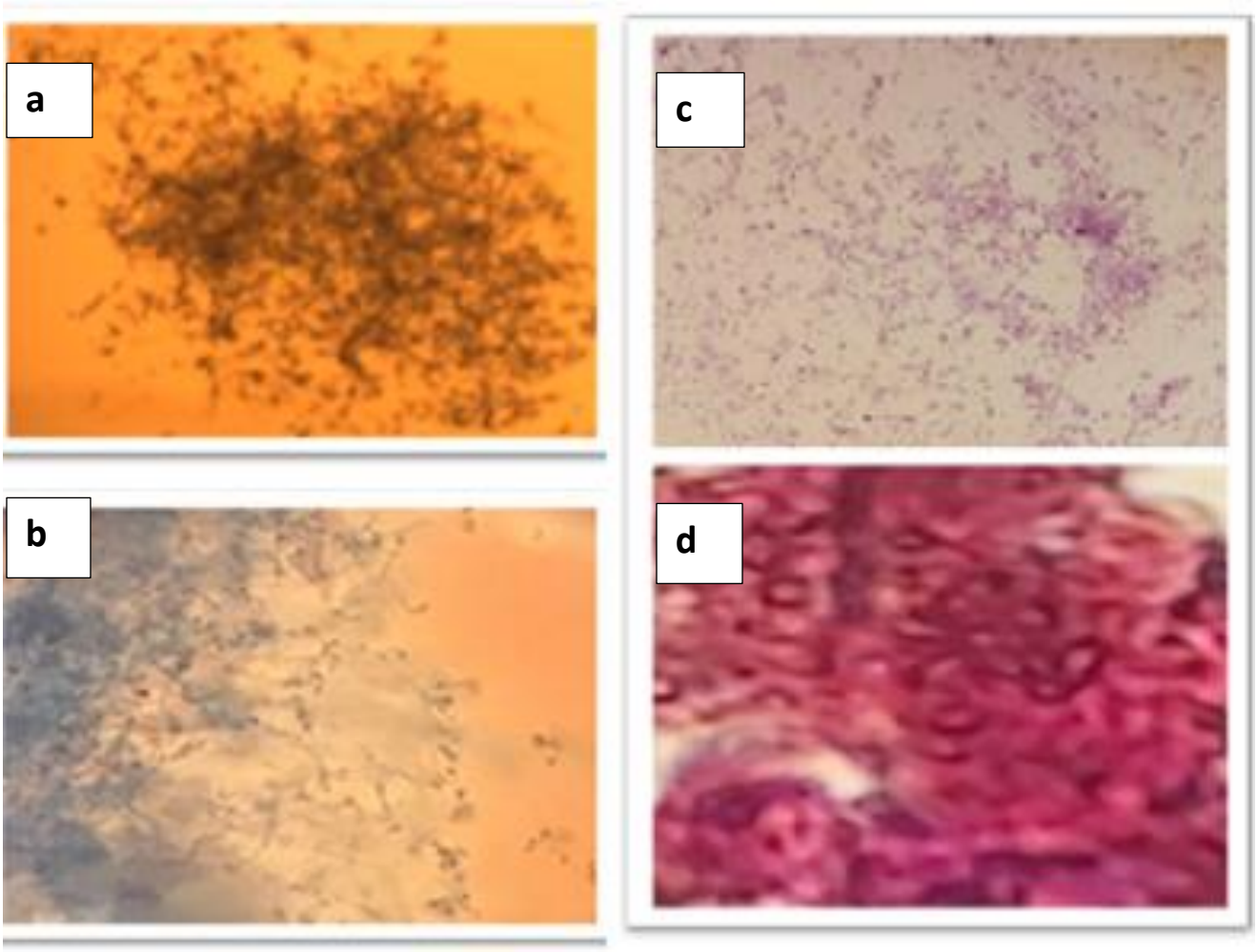


Figure 3.2: a, b, Microscopic view of Isolated actinomycetes *M.lacticum* magnification 40 x. (using cotton blue stain), c, d, Microscopic view of *P.mosselii* Bacteria magnification 100 x. (using gram stain) .

3.3 Molecular Phylogenetic Analysis and Characterization

3.3.1 Agarose Gel Electrophoresis

After amplified DNA by PCR using agarose gel electrophoresis, and document. The result 16S DNA clear bands were at 1500bp in size. the chosen isolate's generate amplicons were detected with a percent identity , the 16S rRNAgene sequence reveal that

the isolated bacteria was comparable to Gen Bank. The nucleotide sequence is deposit in the GenBank database (Abd-Elnaby *et al*,2016).

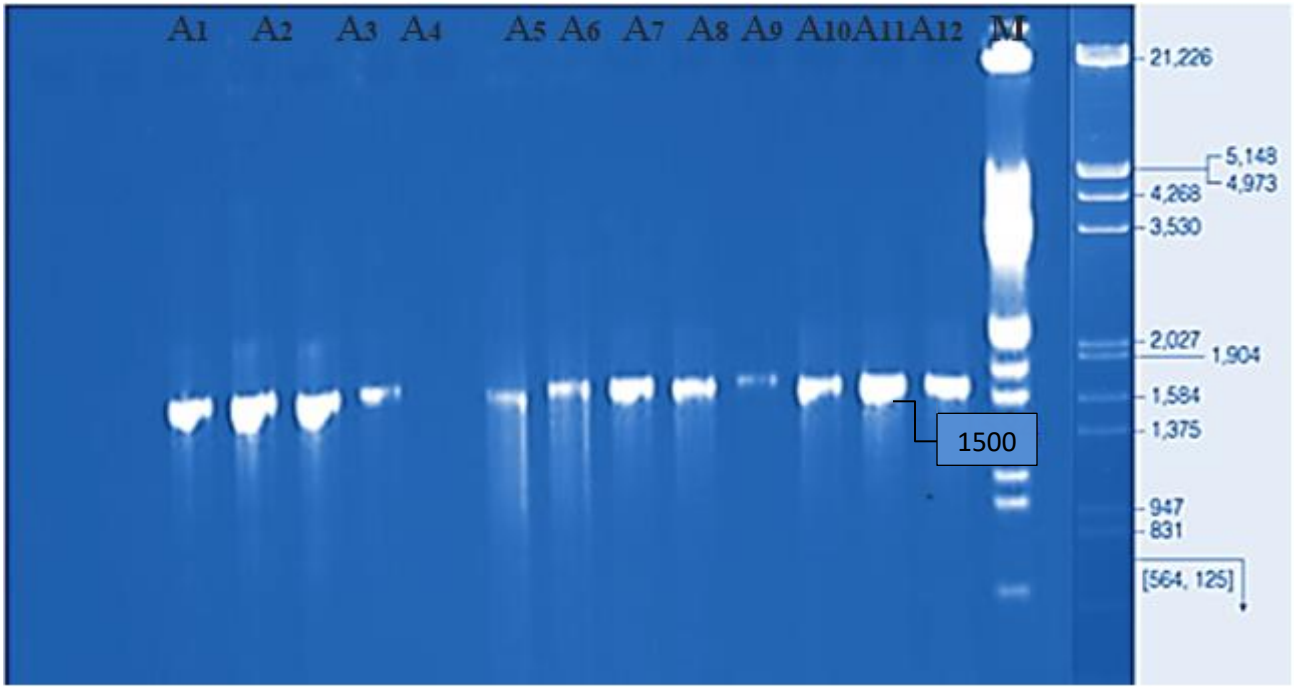


Figure 3.3 Genomic DNA Isolated from Twelve Bacterial Isolates that Electrophorese on 1 Percent Agarose Gel at 80volts, for 80min. Photograph Using a UV Gel Viewer, M:100bp DNA ladder.

Below the accession number of the isolated bacteria in this study, some of these isolated having new accession number because they were new strains so they registered in GenBank, as shown in table (3.2).

Table 3.2:Identification of Isolated Bacteria by 16S rDNA Gene Sequencing

Isolation code	Accession no. of closet species	Closet species	Sequence identity with reference strain %	Accession no. of New strain
A1	KF030881.1	*Ralstonia solanacearum*	83%	MZ703620.1
A2	MF716706.1	*Pseudomonas mosselii*	99%	
A3	KY393059.1	*Microbacterium lacticum*	100%	
A4	MT341804.1	*Ralstonia pickettii*	100%	
A5	KT719711.1	*Microbacterium paraoxydans*	95%	MZ701742.1
A6	MN272363.1	*Pseudomonas koreensis*	99%	MZ768791.1
A7	MT341804.1	*Ralstonia pickettii*	96%	MZ701796.1,
A8	MK241857.1	*Rhizobium pusense*	100%	
A9	CP052129.1	*Ralstonia solanacearum*	93%	MZ701787.1
A10	MN372322.1	*Cupriavidus respiraculi*	98%	MZ768540.1
A11	GU377179.1	*Pseudomonus putida*	100%	
A12	OM362918.1	*Streptomyces Venezuelae*	99%	MZ712042.1

3.3.2 New Strain Registered in GenBank by Using NCBI Online Blast

From the twelve isolates seven were new strain, so they register in GenBank this new strains were:

1.*Streptomyces venezuelae* strain JmKmYa12, identical:100%, sequence ID:MZ712042.1 , length:1391bp.

2.*Ralstonia solanacearum* strain Thuraya 9, identical:100%, sequence ID: MZ703620.1, length 895bp.

3.*Ralstonia solanacearum* strain Mehbas, identical:100% sequence ID: MZ701787.1 , length:708bp.

4. *Ralstonia pickettii* strain Dewan7, identical:100%, sequence ID: MZ701796.1, length:783bp.

5. *Microbacterium paraoxydans* strain shahooda, identical: 100% sequence ID: MZ701742.1, length:1388bp.

6. *Pseudomonas koreensis* strain Allawy, identical:100%, sequence ID: MZ768791.1, length1419bp.

7. *Cupriavidus respiraculi* strain Halema, identical:100%, sequence ID: MZ768540.1, length:1398bp.

3.3.3 Other Strains Already in GenBank

The rest of the five isolate were already in the GenBank:

1.*Pseudomonas Mosselii* strain XG1-1-2, identical:100%, sequence ID:MF716706.1, length:1419bp.

2. *Microbacterium lacticum* strain STM54, identical:100%, sequence ID: KY393059.1, length:949bp.

3. *Rhizobium pusense* strain WTB70, identical:100%, sequence ID:MK241857.1 length:1390bp.

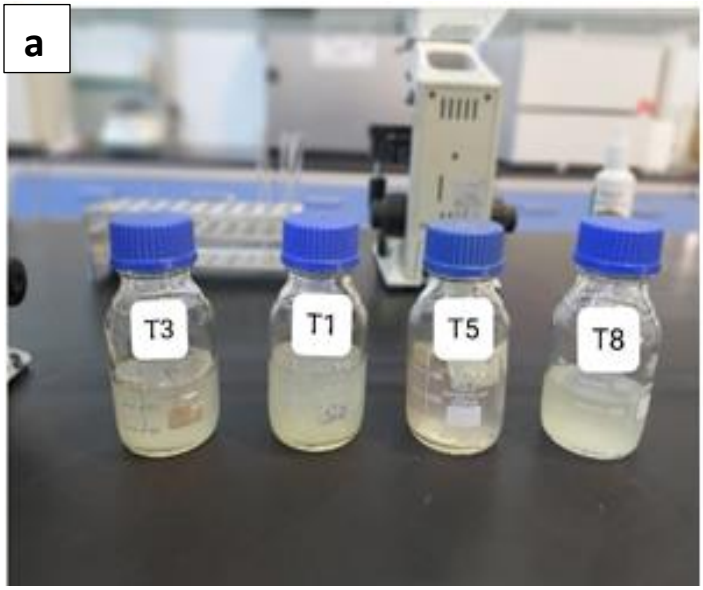
4. *Ralstonia pickettii* strain CHP10, identical:100%, sequence ID: MT341804.1, length:844bp.

5.*Pseudomonas putida* strain LW, identical:100%, sequence ID: GU377179.1, length:1445bp.

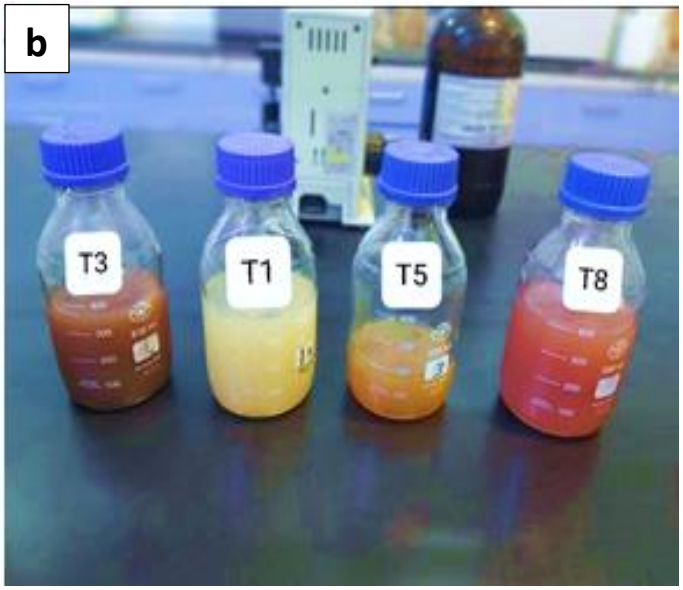
3.4 Production of Extracellular AgNPs in Laboratory

After 7days the reaction mixture are bio-synthesize AgNPs, changes in color from translucent white to reddish brown, depending on the synthesis strain indicating that the bacteria synthesize AgNPs, This means that the isolated bacteria had the enzyme

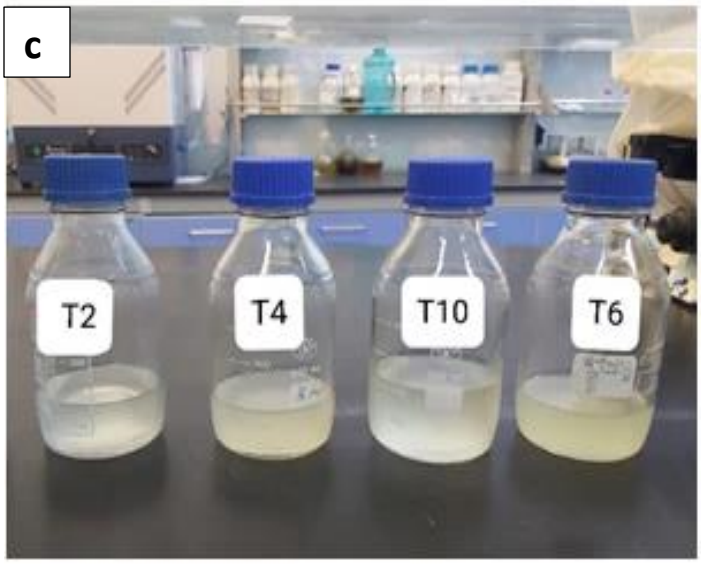
(nitrate reductase) produced by cell activities that target metal ions from their environment and bio-reduction silver to convert Ag^{+} to Ag^{0} followed by increase in the kinetics of the deposition of the silver atoms, resulting the extracellular creationof AgNPs (Elbeshehy,*et,al.*,2015).



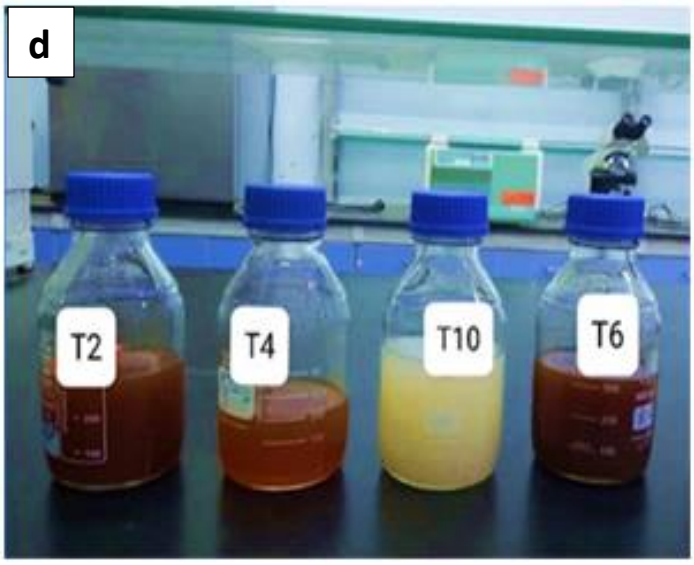
a-The supernatant with out AgNO3.



b- the supernatant containing AgNO3.



c- The supernatant with out AgNO3.



d- the supernatant containing AgNO3.

Figure 3.4: a, b, c, d, Extracellular Synthesis of AgNPs by Isolation Bacteria after 7 days.

As shown in figure (3.4), the supernatant of *P.mosselii* after incubation period change in color from translucent white to light brown (sample T1), while the changed color of *M. lacticum* supernatant from translucent white to reddish brown (sample T2), the supernatant of *P.koreensis* change from translucent white to dark brown (sample T3), the supernatant of *R.pickettii* strain Dewan7 color change from translucent white to reddish brown (sample T4), while the color change of *R.pusense* from translucent white to brown (sample T5), the supernatant of *R.pickettii* strain CHP10 change from translucent white to reddish brown (sample T6), while the color change of *C.respiraculi* supernatant from translucent white to dark pink (sample T8), and the supernatant of *S.venezuelae* change color from translucent white to light brown (sample T10).

In this experiment, the biomass did not produce AgNPS by intracellular biosynthesis,

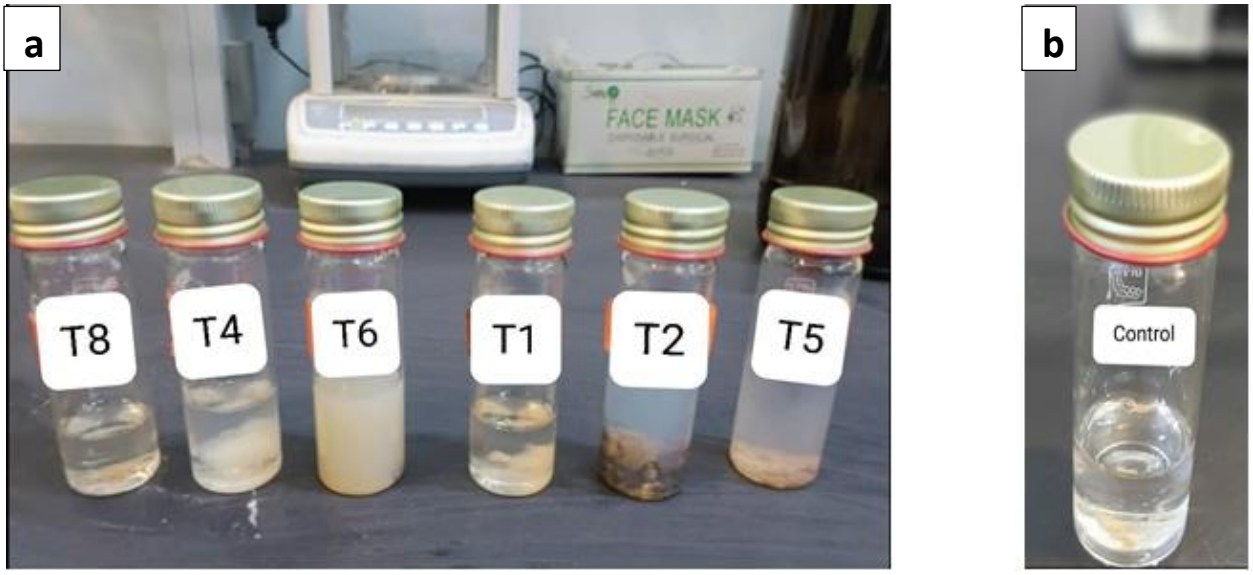


Figure 3.5 Intracellular Synthesis of AgNPs by Isolated Bacteria. a-Biomass with AgNO3 after 7 Days Incubation with out color change. b- Biomass without AgNO3.

3.4.1 Drying of Nanoparticles Solution

After that, the nanoparticle solutions were put in plates (pyrex glass) for drying at 60 ^{o}C for 30 hours, Then scratched the nanoparticles after drying and the powder retained in plates, for further analysis, figure (3.6).

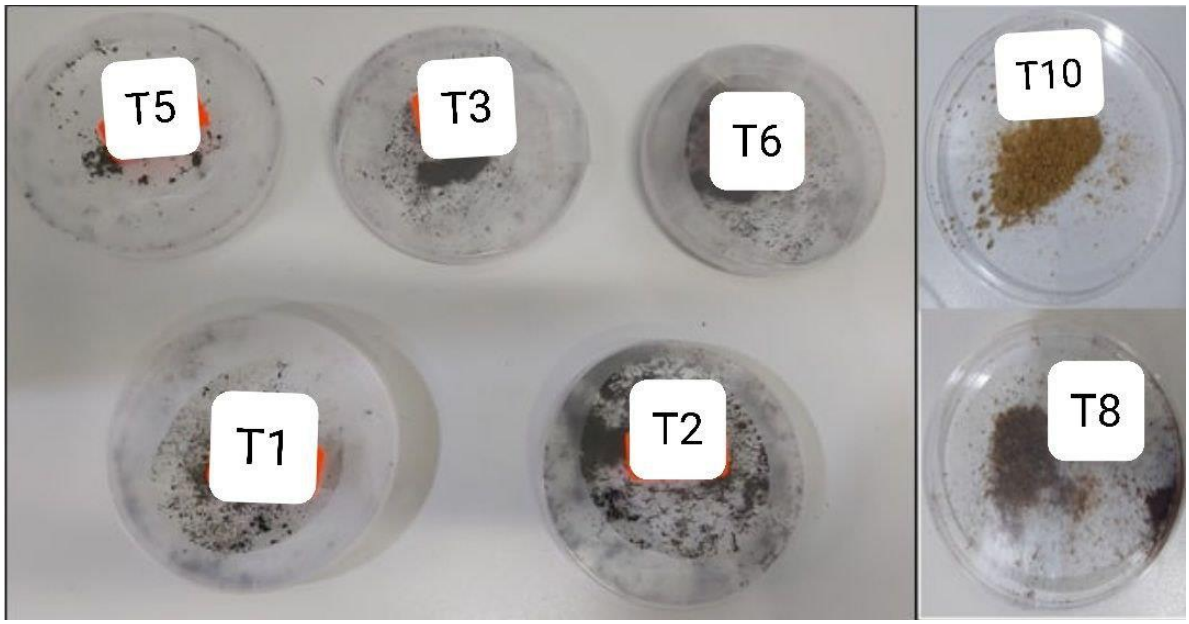


Figure 3.6: Silver Nanoparticles Powder After Scratching From the Plates

3.5 Characterization of Silver Nanoparticles

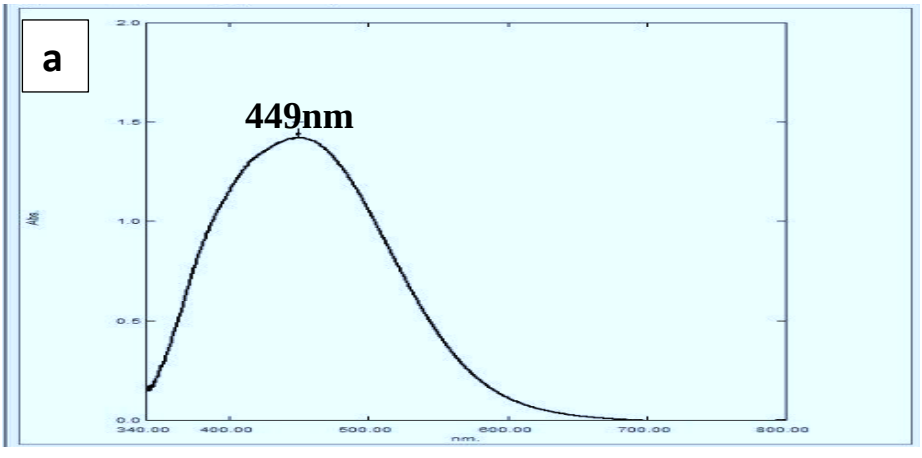
3.5.1 Physical Characterization of AgNPs

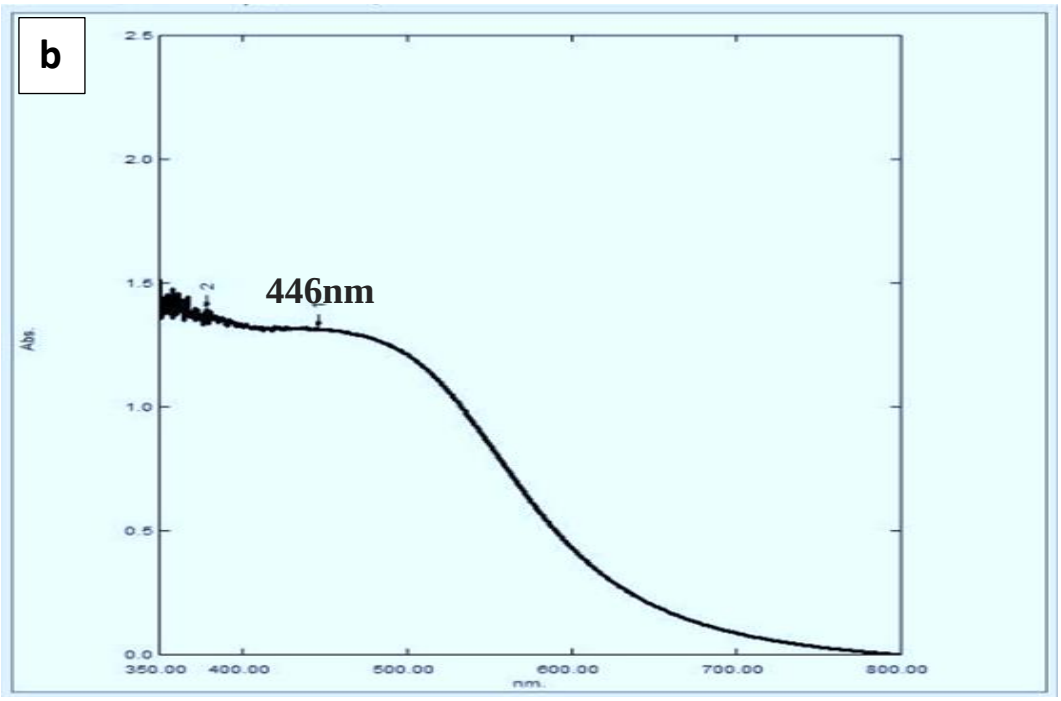
3.5.1.1UV-Vis.Spectroscopy

The intense color of the distributed silver nanoparticles was due to the absorption of the surface plasmon resonance spectrum (SPR) of silver nanoparticles.

Metallic bio nanoparticles, as a result, have a distinct optical absorption spectrum in the UV visible region, and exhibit a distinct optical absorption spectrum in the UV-visible range. AgNPs have a significant UV–visible spectrum, as shown in figure (3.7- a, b, c, d,

e,).The best 5 synthesized AgNPs (a, b, c, d, e) were chosen, the wave lengths are very good, broad peak of them between (444-489 nm). In figure (3.7-a), represented silver nanoparticles synthesize laboratory using *Streptomyces venezuelae* strain JmKmYa12, the broad peak of this nanoparticles (449) nm wavelength at the absorption value (1.4), While silver nanoparticles synthesize by *P. putida* strain LW shown wavelength (446) nm at the absorption value (1.3) as shown in figure (3.7-b).The wavelength of silver nanoparticles that fabricate by bacteria *C. respiraculi* strain Halema is (444) nm at the absorption value (1.7) as shown in figure (3.7-c), While silver nanoparticles synthesize by *R.pickettii* strain CHP10 shown wavelength (471) nm at the absorption value (1.2) as shown in figure (3.7-d). The wavelength of silver nanoparticles that fabricate by bacteria *M.lacticum* strain STM54 is (489) nm at the absorption value (0.2) as shown in figure (3.7-e). While the non-treatment supernatant with AgNO3 that use as a Control doesn't show any absorption peak on UV-vis spectroscopy this agreed with previous studies (Huq,2020).The UV-Vis spectroscopy an analytical technique to the detect of AgNPs in solutions because of the optical characteristics of nanoparticles and resulting from SPR (Othman *et al.*,2019) and the absorption of specific selected volume is related to the size of the size of the naturally the size and dimensions of the nanoparticles. The UV-vis. spectroscopy highly absorption to the large part of SPR and the determination of the bio-reduction of silver nanoparticles as well as the determination of the size of nanoparticles by determining the absorption sites at wavelengths, as well as the increase in the size of the nanoparticles leads to the transition of the SPR peak to a longer wavelength. (Prathna *et al.*,2011)

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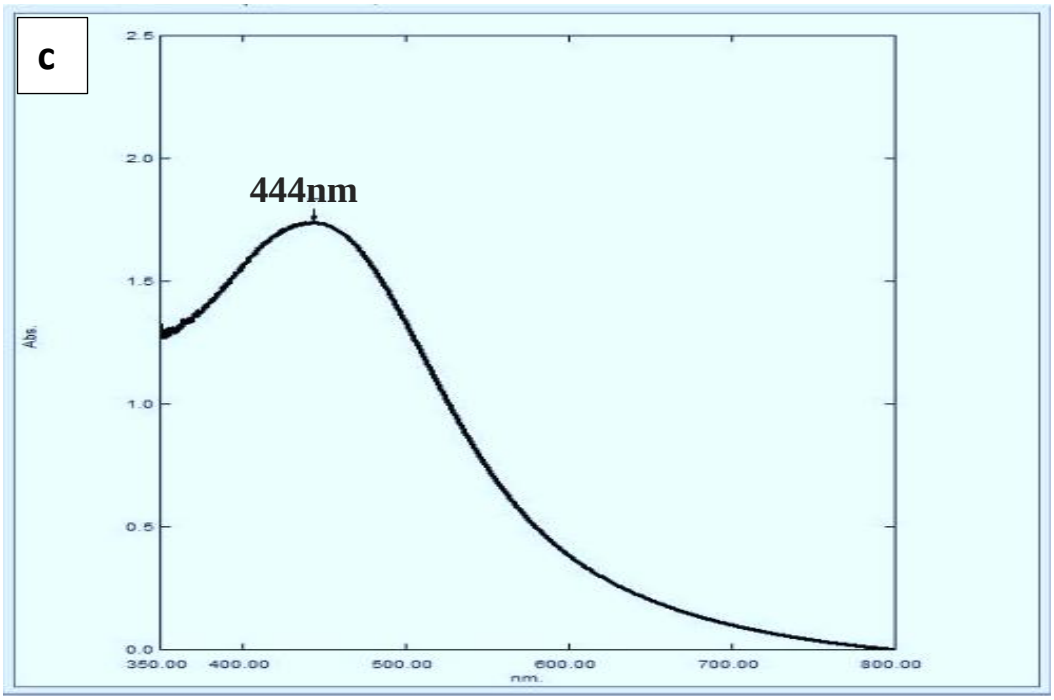
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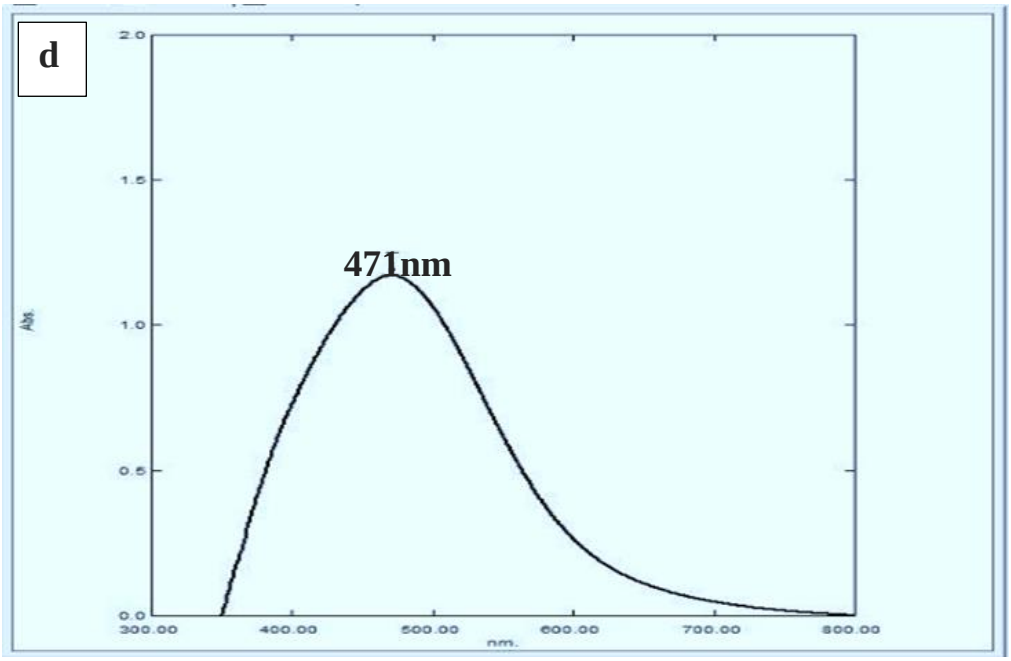


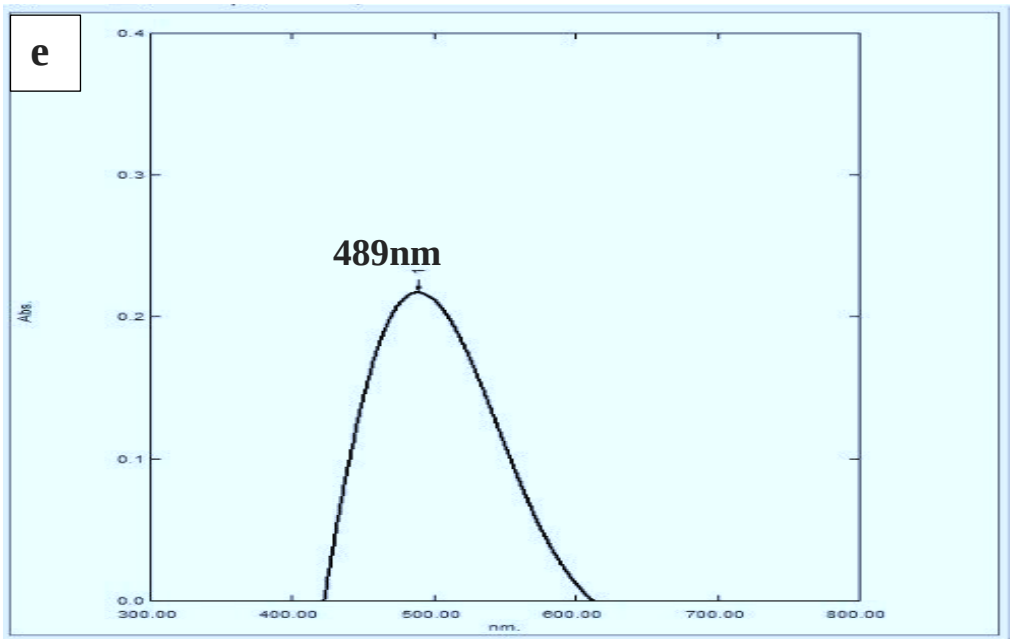


Figure 3.7: UV-vis. spectroscopy of silver nanoparticles synthesize laboratory using(a-*S. venezuelae.* b- *R.putida*. c- *C.respiraculi.* d- *R.pickettii*. -e- *M.lacticum)*.

This results of UV-vis. spectroscopy were shown in table (3.3).

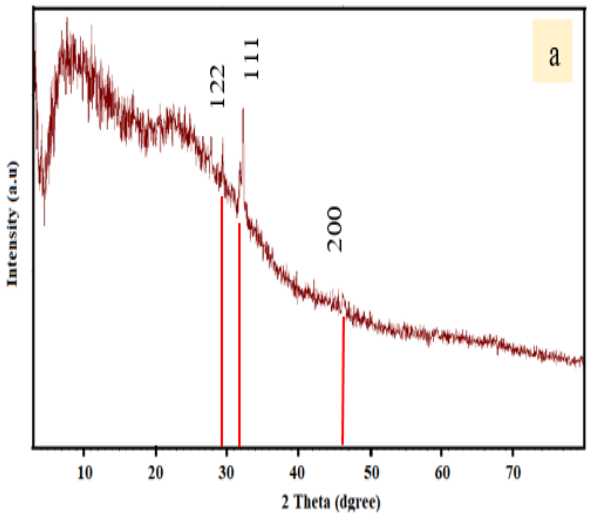
Table 3.3: UV-vis. Spectroscopy of Different Bacteria Synthesized AgNPs

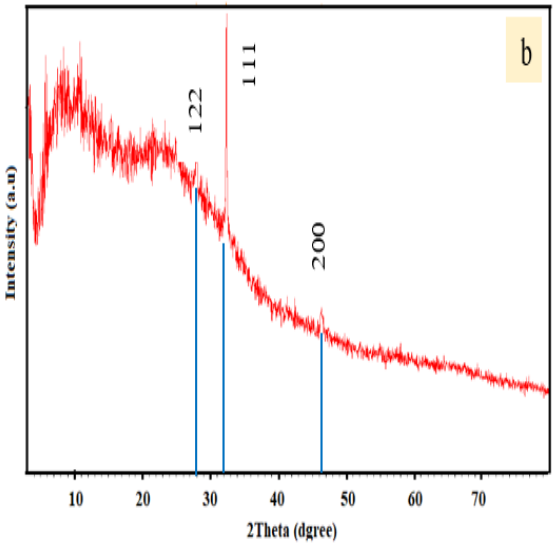
ID Samples	Type of AgNPs Synthesized Bacteria	Wave Length
a	***S. venezuelae***	**449 nm**
b	***R.putida***	**446 nm**
c	***C.respiraculi***	**444 nm**
d	***R.pickettii***	**471 nm**
e	***M.lacticum***	**489 nm**

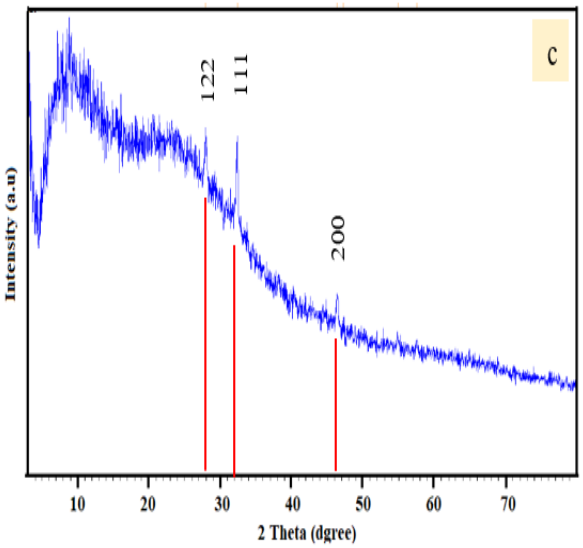
3.5.1.2 XRD Analysis

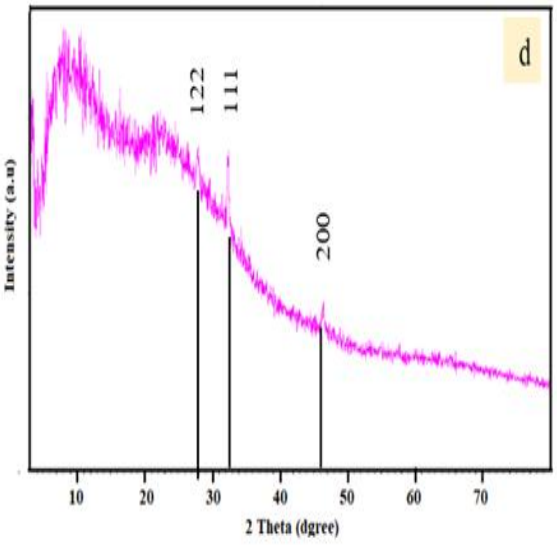
Figure (3.8) analysis of structure and crystalline size of the silver nanoparticles were carried out by XRD. The XRD analysis of silver nanoparticles showed diffraction peaks at 2θ = 32.5°, 28.3°, and 48.1° respectively. When compared with the standard, the obtained XRD spectrum confirmed that the silver nanoparticles were in nanocrystal form and crystalline. The peaks can be corresponding to values planes (122), (111) and (200), facet of silver crystal, respectively. Diffraction Standards (JCPDS), silver file No. (04–0783). The XRD study confirms / indicates that the resultant particles are face-centered cubic (FCC) silver nanoparticles. The high peaks in the XRD analysis indicated the active silver composition with the indexing. Like this, no any spurious diffraction which indicating the crystallographic impurities in the sample. All the reflections correspond to pure silver metal with face centered cubic symmetry. The high intense peak for FCC materials is generally (111) reflection, which is observed in all samples. The intensity of peaks reflected the high degree of crystallinity of the silver nanoparticles. However, the diffraction peaks are broad which indicating that the crystallite size is very small. The

observe peak broadening and noise were probably macromolecules present, which may be responsible for the reduction of silver ions. Hence, XRD pattern thus clearly illustrated that the silver nanoparticle form in this present were crystalline. The XRD shows that silver nanoparticles formed were crystalline and indicates that the silver nanoparticles were face-centered cubic (Hassan,2018).









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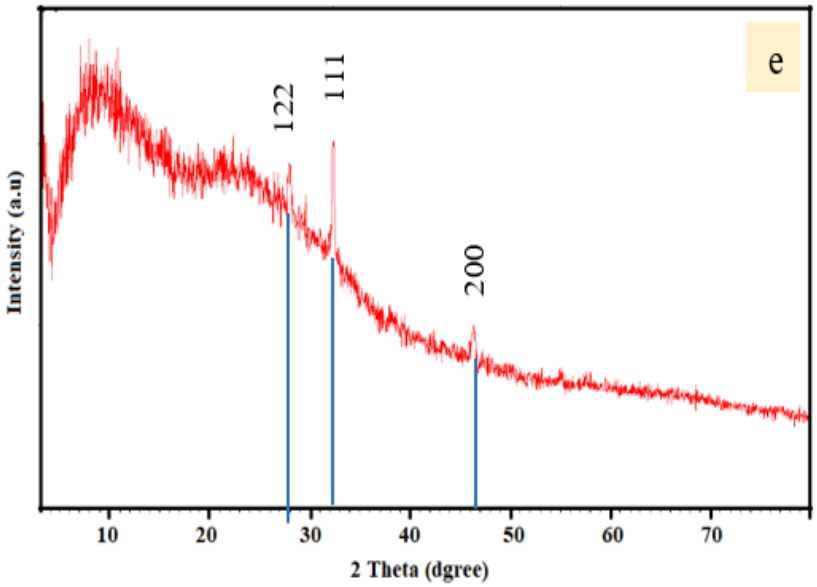


Figure 3.8: XRD of AgNPs synthesize by :(a-*S. venezuelae*. b- *R.putida.* c- *C.respiraculi.* d- *R. pickettii* e- *M.lacticum).*

3.5.1.3 TEM Analysis

The size and morphology of particles were assessed using a TEM, micrograph shows silver particles with an average diameter of about 50–100 nm of well-dispersed and predominantly spherical in shape as shown in figure(3.9). Moreover, the nanoparticles were well disperse without significant agglomeration or variations in morphology of all samples. The present data were in agreement with (Singh *et al*,2008). TEM images show that the AgNPs were with a narrow size distribution, which corresponds to the shape of UV-visible spectra. Some agglomerates can be observe. This indicate formation of mono-dispersed silver nanoparticles with spherical shape. Micrograph of synthesized nanoparticles at 100 nm magnification indicates an average size ranging from 22-28nm and was spherical in shape.

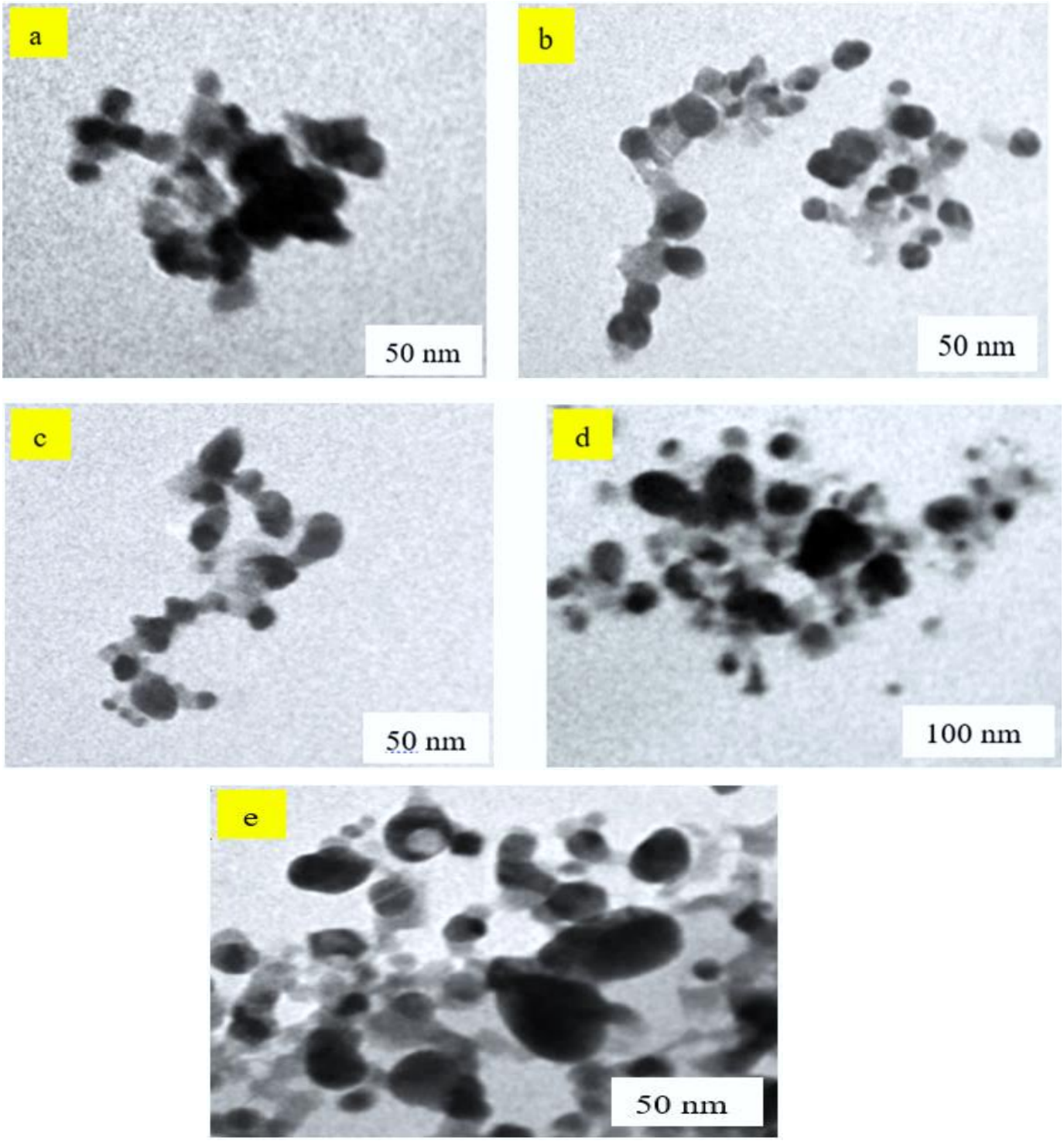
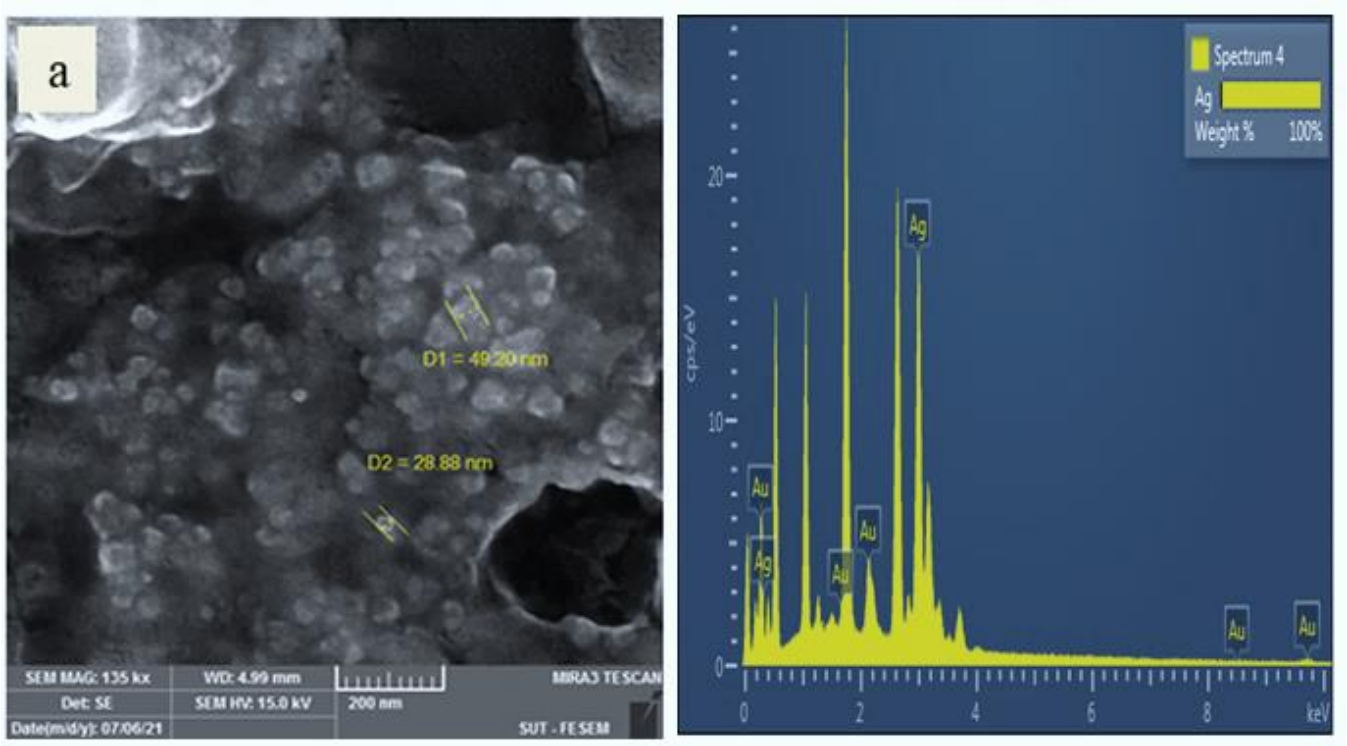
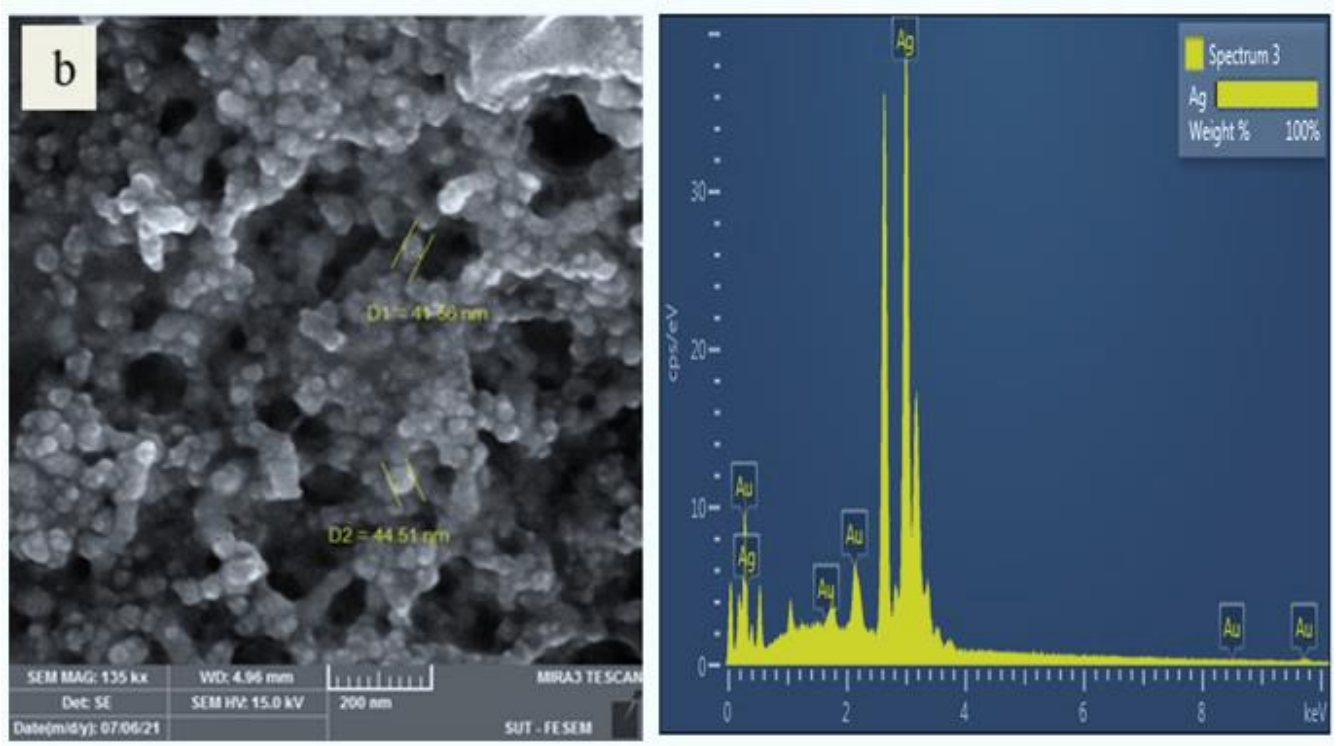


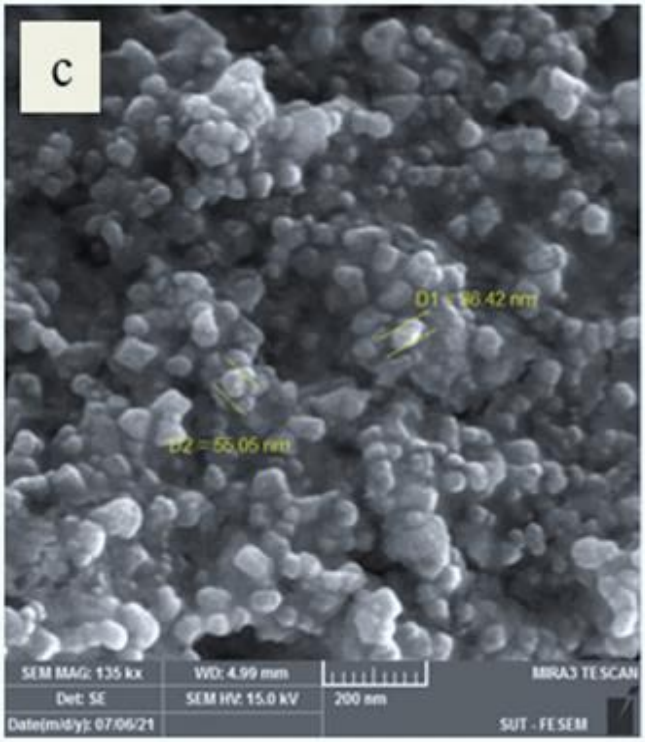
Figure 3.9: TEM of AgNPs synthesize by (a-*S.venizuelae.* b- *P.putida*. c- *C.respiraculi.* d- *R.pickettii*. e- *M.lacticum)*.

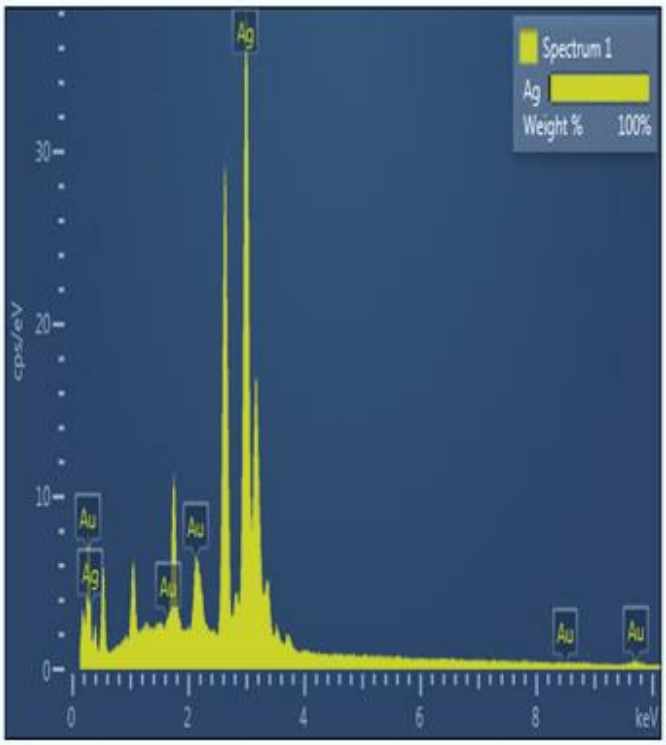
3.5.1.4 SEM and EDX Analysis

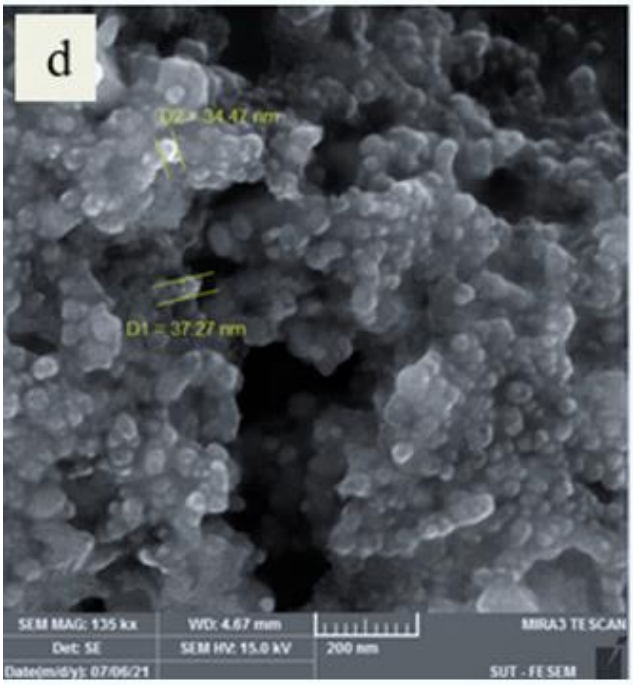
SEM analysis shows high-density AgNPs (figure 3.10). It was shown that relatively spherical and uniform AgNPs were formed with diameter of 20 to 70 nm. The SEM image of silver nanoparticles was due to interactions of hydrogen bond and electrostatic interactions between the bioorganic capping molecules bound to the AgNPs. The nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a capping agent . The larger silver particles maybe due to the aggregation of the smaller ones, due to the SEM measurements. The grain sizes of the samples estimated from the SEM picture is larger than that obtained from XRD data. This means that, the SEM picture indicates the size of polycrystalline particles. Generally, on the Nano meter scale, metals (most of them are fcc) tend to nucleate and grow into twinned and multiply twinned particles (MTPs) with their surfaces bounded by the lowest-energy [111] facets. The observation of some larger nanoparticles maybe attributed to the fact that Ag nanoparticles have the tendency to agglomerate due to their high surface energy and high surface tension of the ultrafine nanoparticles. The fine particle size results in a large surface area that in turn. The energy dispersive spectroscopy X-ray (EDX) data show very strong silver peaks (figure 3.10), this results agree with other researchers, (Kasithevar, *et al*, 2017).the size of silver nanoparticles that synthesize by *S.venizuelae* range from 28-49nm (sample a), while the size of silver nanoparticles that synthesize by *P.putida* range from 41-44nm (sample b), the size of silver nanoparticles that synthesize by *C.respiraculi* range from 36-55nm (sample c), the size of silver nanoparticles that synthesize by *R.pickettii* range from 34-37nm (sample d), and the size of silver nanoparticles that synthesize by *M.lacticum* range from 20-70nm (sample e).

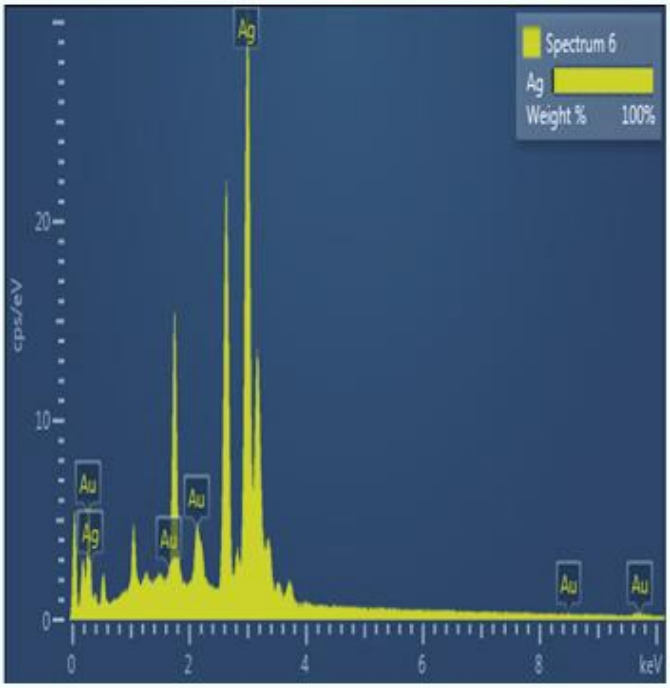

a
D1 = 49.20 nm
D2 = 28.88 nm
SEM MAG: 135 kx
WD: 4.99 mm
MIRA3 TESCAN
Det: SE
SEM HV: 15.0 kV
200 nm
Date(m/d/y): 07/06/21
SUT - FESEM
Spectrum 4
Ag
Weight %
100%
cps/eV
keV
Au
Ag


b
D2 = 44.51 nm
SEM MAG: 135 kx
WD: 4.96 mm
MIRA3 TESCAN
Det: SE
SEM HV: 15.0 kV
200 nm
Date(m/d/y): 07/06/21
SUT - FESEM
Spectrum 3
Ag
Weight %
100%
cps/eV
keV
Au
Ag


c
D1 = 36.42 nm
D2 = 58.05 nm
SEM MAG: 135 kx
WD: 4.99 mm
Det: SE
SEM HV: 15.0 kV
200 nm
MIRA3 TESCAN
Date(m/d/y): 07/06/21
SUT - FE SEM


Spectrum 1
Ag
Weight % 100%
cps/eV
keV
Ag
Au
Au
Ag
Au
Au
Au


d
D2 = 34.47 nm
D1 = 37.27 nm
SEM MAG: 135 kx
WD: 4.67 mm
Det: SE
SEM HV: 15.0 kV
200 nm
MIRA3 TESCAN
Date(m/d/y): 07/06/21
SUT - FE SEM


Spectrum 6
Ag
Weight % 100%
cps/eV
keV
Ag
Au
Au
Ag
Au
Au
Au

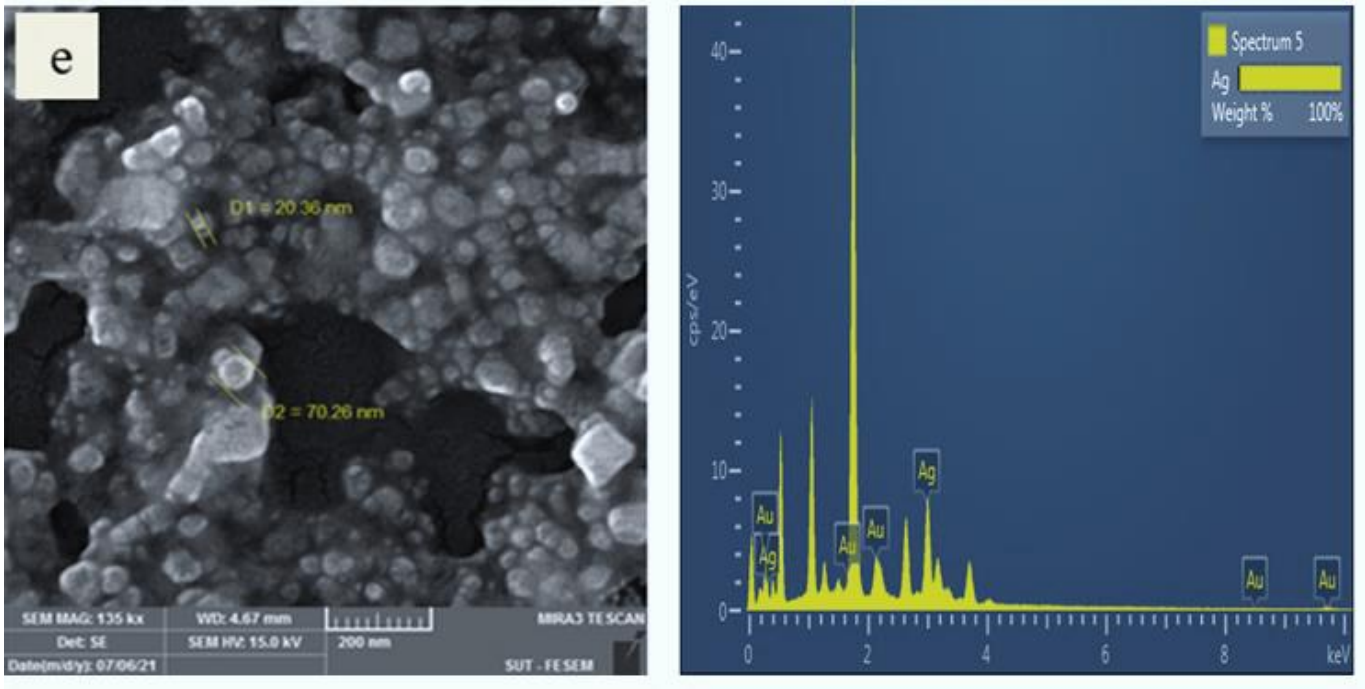


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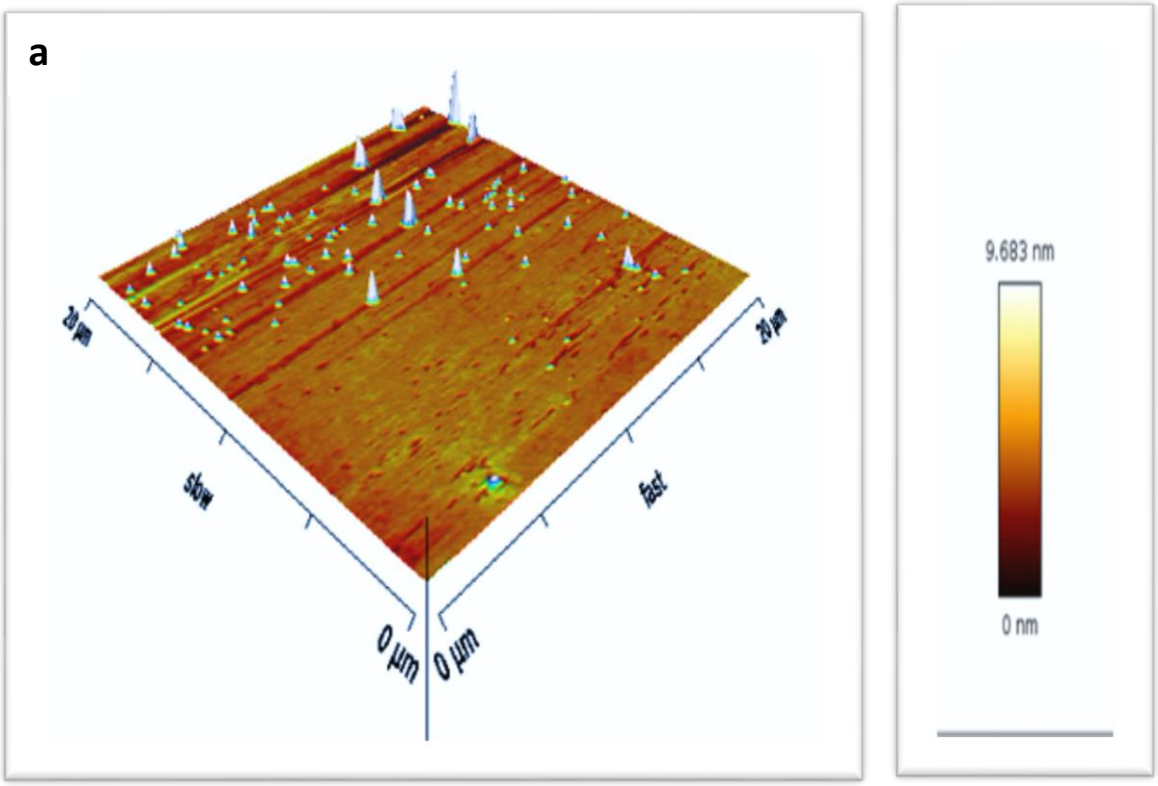
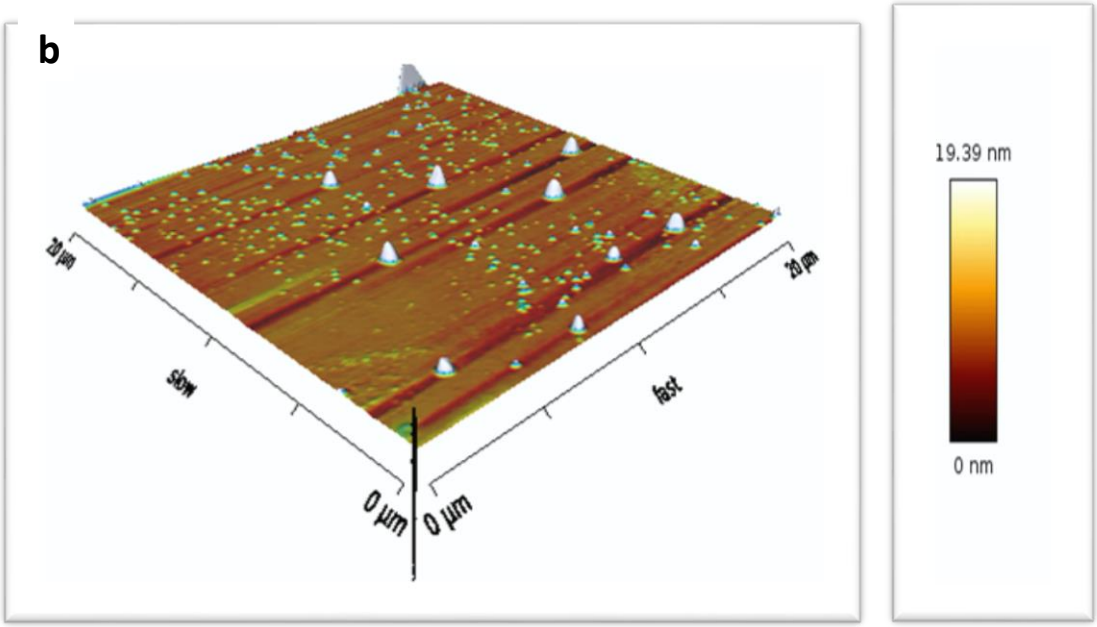
Figure 3.10: SEM, EDX of AgNPs synthesize by (a-*S.venizuelae*. b- *P.putida*. c- *C.respiraculi.* d- *R.pickett.* e- *M.lacticum)*.

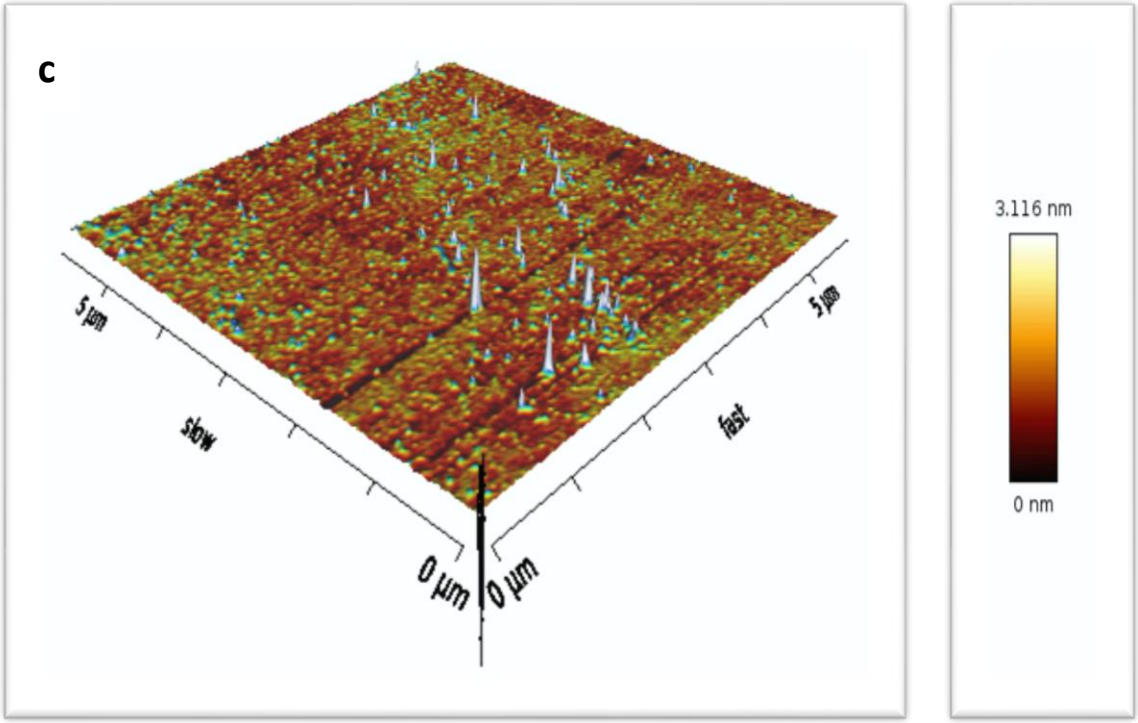
which indicate that the reduction of silver ions to elemental silver possibly originated from the molecules attached to the surface of the AgNPs. The dense peak of silver strongly confirmed the reduction of silver nitrate to silver nanoparticles. Silver peak was thicker than other peak. This confirms the complete reduction of silver compounds to AgNPs as shown in the spectrum. The EDX reading proved that the required phase of silver (Ag) is present in the samples. Generally, silver Nano crystals demonstrate typical optical absorption peak.

3.5.1.5 AFM Analysis

The shape, average diameter, and roughness of AgNPs generated by the 5 strains have be en described using an atomic force microscope image. To control the shape and size of the finished structure, both etching time and current density were use. The average diameter of AgNPs fabricate by *S.venezuelae* is 9.683 nm figure (3.11-a), average diameter of AgNPs fabricate by *P.putida* is 19.39 nm figure (3.11-b), average diameter of AgNPs fabricate by *C.respiraculi* was 3.116nm figure (3.11-c),while those fabricate by *R.pickettii* is 3.392 nm figure (3.11-d), and the average diameter of biogenic AgNPs fabricate by *M.lacticum* is 32.44nm figure (3.11-e).




b
20 µm
slow
fast
20 µm
0 µm
0 µm
19.39 nm
0 nm


c
5 µm
slow
fast
5 µm
0 µm
0 µm
3.116 nm
0 nm

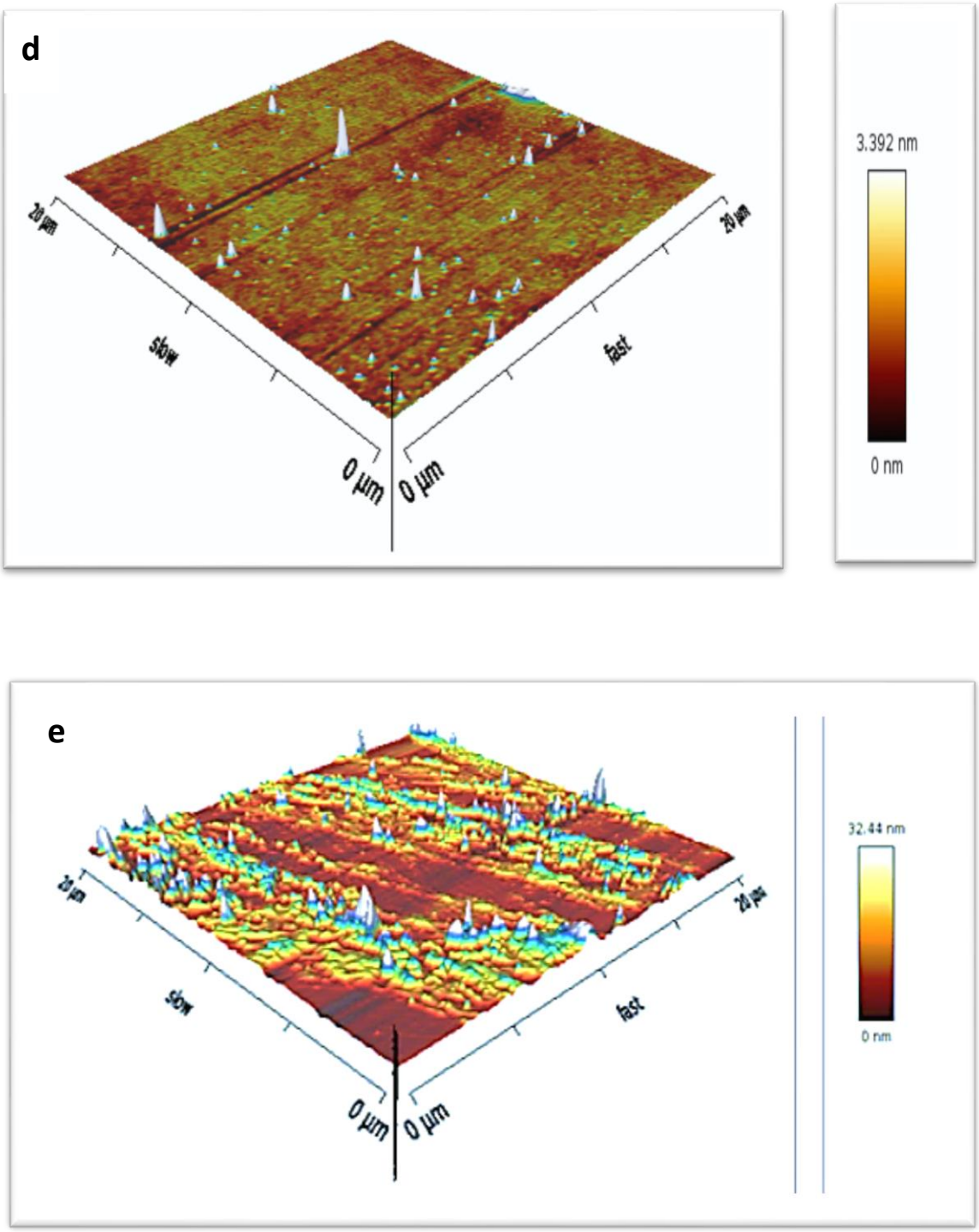


Figure 3.11: AFM of AgNPs fabricate by (a-*S.venezuelae.* b- *P.putida.* C- *C.respiraculi.* d- *R.pickettii.* e- *M.lacticum*).

3.5.2 Biological Characterization of AgNPs

3.5.2.1Antibacterial Activity of AgNPs

The antimicrobial activity of biosynthesized AgNPs were studied against ten species of pathogenic bacteria isolated from the hospital as shown in table (3.4).

Table 3.4: Pathogenic Bacteria Use in this Experiment.

NO.	Pathogenic bacteria	Samples Source
1	*Staphylococcus aureus*	Ear
2	*Staphylococcus haemolyticus*	Wounds
3	*Staphylococcus hominis*	Nipple discharge
4	*Escherichia coli*	Urine
5	*Pseudomonas aeruginosa*	Burns
6	*Klebsiella pneumonia*	Urine
7	*Salmonella typhi*	Blood
8	*Enterobactor cloacae*	Wounds
9	*Staphylococcus lentus*	Urine
10	*Proteus mirabilis*	Wounds

The antimicrobial activity of AgNPs compare to that of antibacterial medicines such as Gentamicin (in this research),(Wei *et al* 2007).The antibacterial activity of the produce AgNPs demonstrate against all of the ten tested bacteria. The sizes of bacterial inhibition zones vary from pathogenic bacteria to another, and from synthesized AgNPs to another, table (3.5).

Table3.5: Antibacterial Activity of Biosynthesized AgNPs Against Pathogenic Bacteria Isolated from the Hospital

Synthesized AgNPs and Gentamycin	Inhibition zone (mm) on Pathogenic Bacteria									
	S. aureus	*S. haemolyticus*	*S. hominis*	*S. lentus*	*P. aeruginosa*	*K. pneumoniae*	*S. typhi*	*E. cloacae*	*E. coli*	*P. mirabilis*
T1	26.2	14.7	49	12.7	37	9.0	35.5	11.5	33	39.5
T2	38	22.5	7.0	21	17	18	33.5	12	9.5	10
T3	29	38.5	10	19.5	40	11.5	28.5	10.5	32.3	39.5
T4	12.5	11.5	11	10.7	38.5	16.5	15	7.7	35	6.0
T5	20.5	12	11.5	17	21.5	15	12	11.5	11.5	24
T6	14.5	12.5	12.7	11.5	14.5	9.5	10.5	15.5	34.5	28.5
T7	28.3	18.5	20	17	15.5	13.5	14	16	28.5	31
T8	12.5	20.5	11	9.5	13	13.5	21	23.5	11	8.0
T9	19.5	13	47.3	26.3	36.5	16.5	27.5	13	36.5	6.0
T10	18.5	13	14.5	14	16.5	16	23	6.0	13.5	17
Gentamycin.	9.0	24.5	13.5	28	13	16.5	24.5	6.0	14	24.5

Below the tested plates of pathogenic bacteria and the inhibition zone by different types of AgNPs biosynthesized from different bacteria.

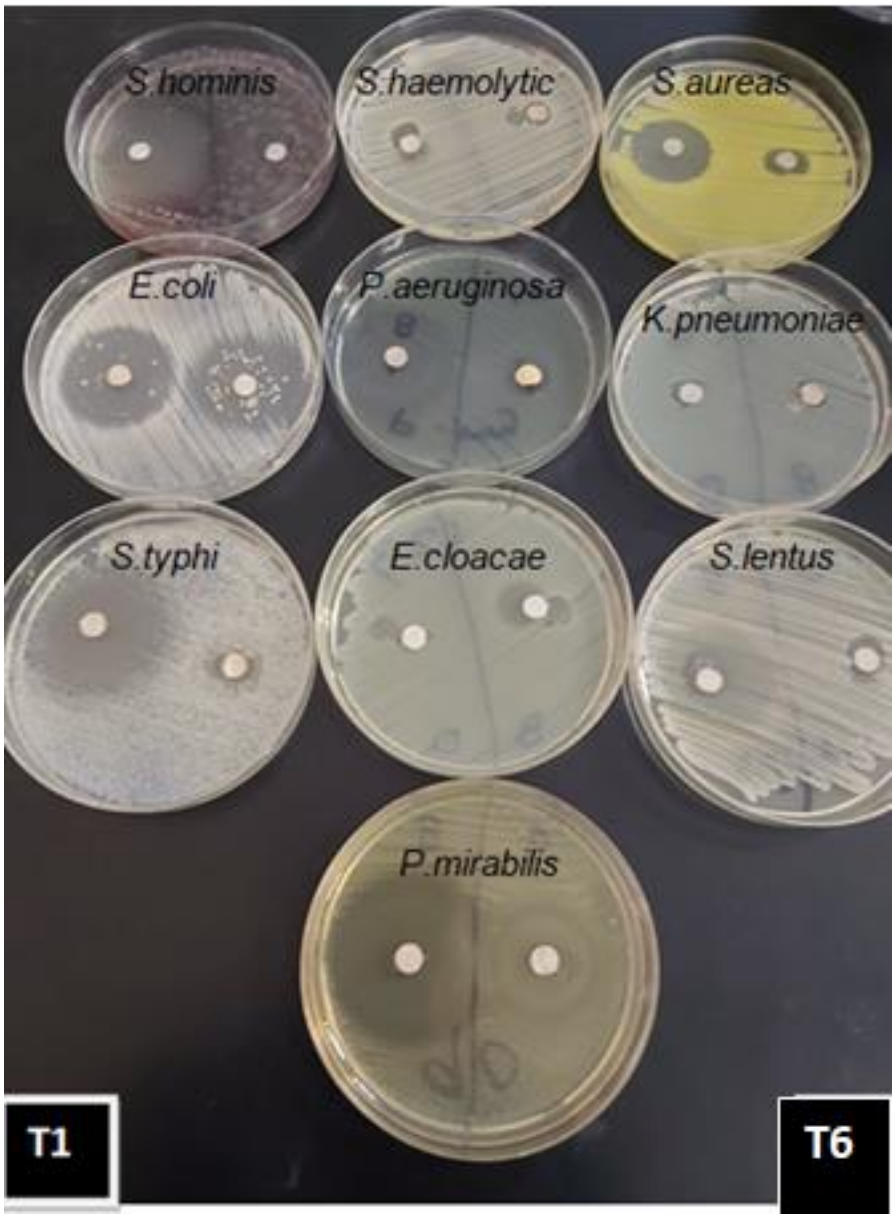


Figure 3.12 Inhibition Zone on ten Pathogenic Bacteria by Biosynthesis AgNPs from T1 (*P. mosselii*) and T6 (*R.pickettii*).

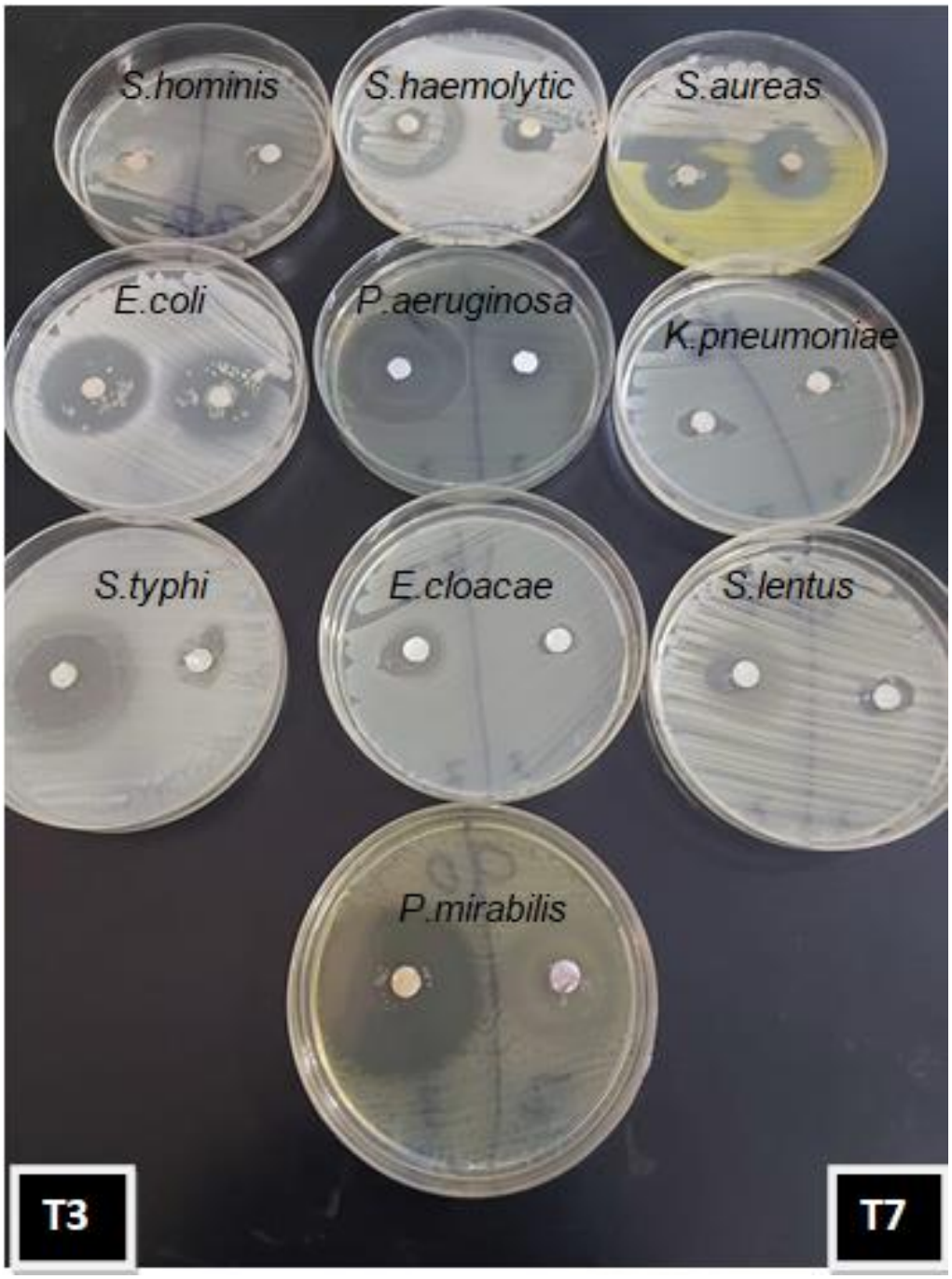


Figure3.13 Inhibition Zone on ten Pathogenic Bacteria by Biosynthesis AgNPs from T3 (*P.koreensis*) and T7 (*R.solanceaum*).

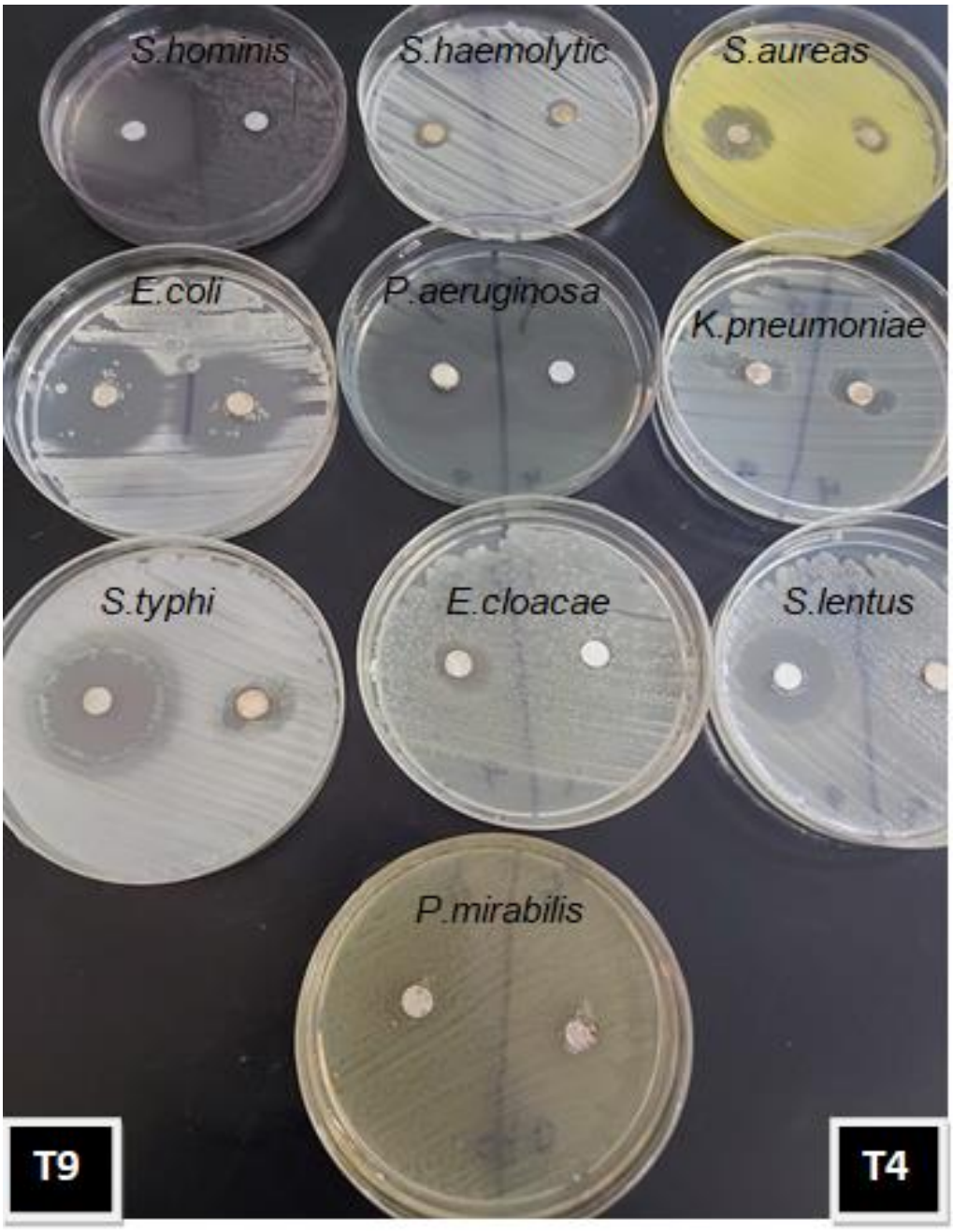


Figure 3.14 Inhibition Zone on ten Pathogenic Bacteria by Biosynthesis AgNPs from T9 (*P.putida*) and T4 (*R.pickettii*).

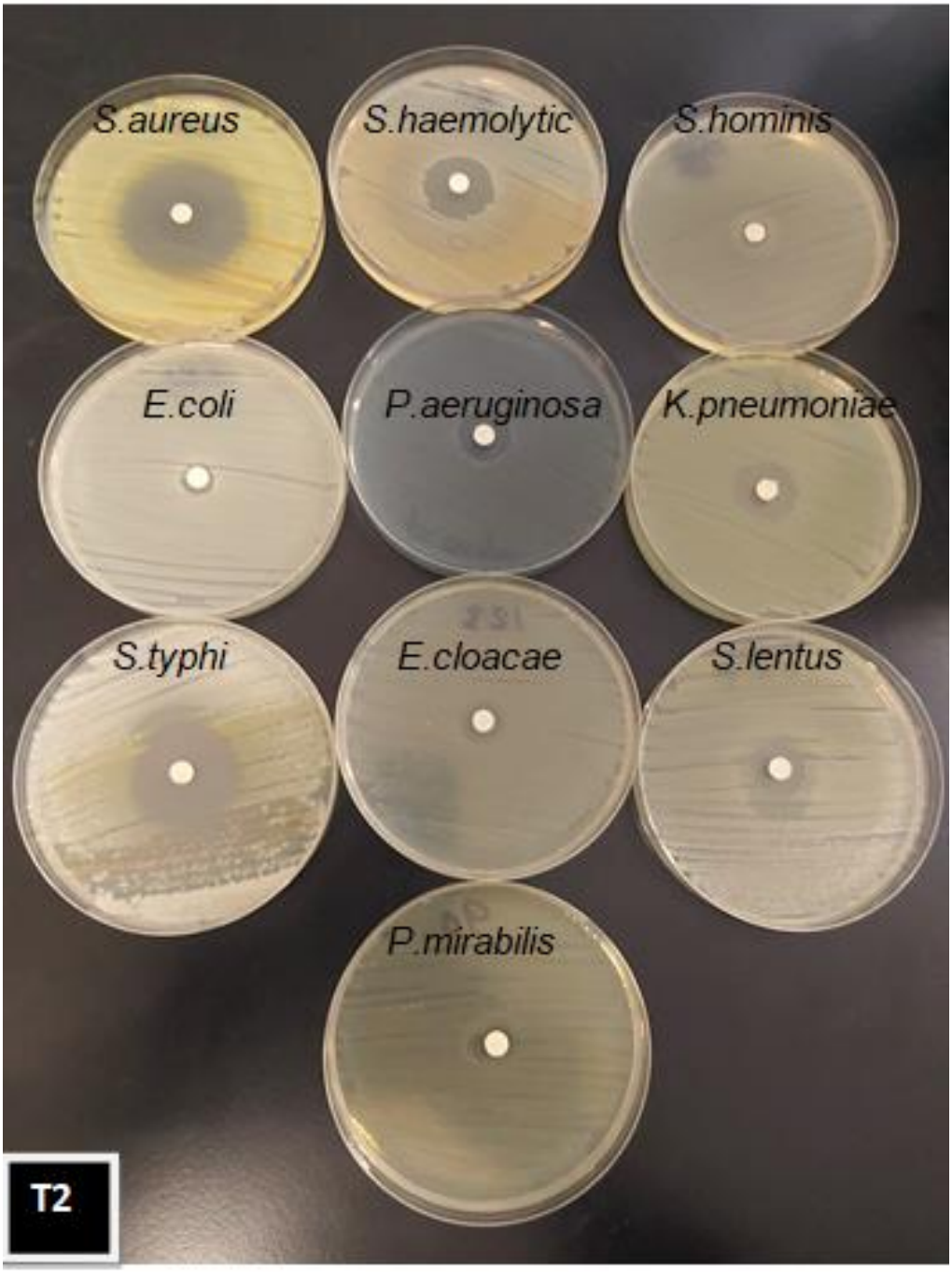


Figure3.15 Inhibition Zone on ten Pathogenic Bacteria by Biosynthesis AgNPs from *M.lacticum*.

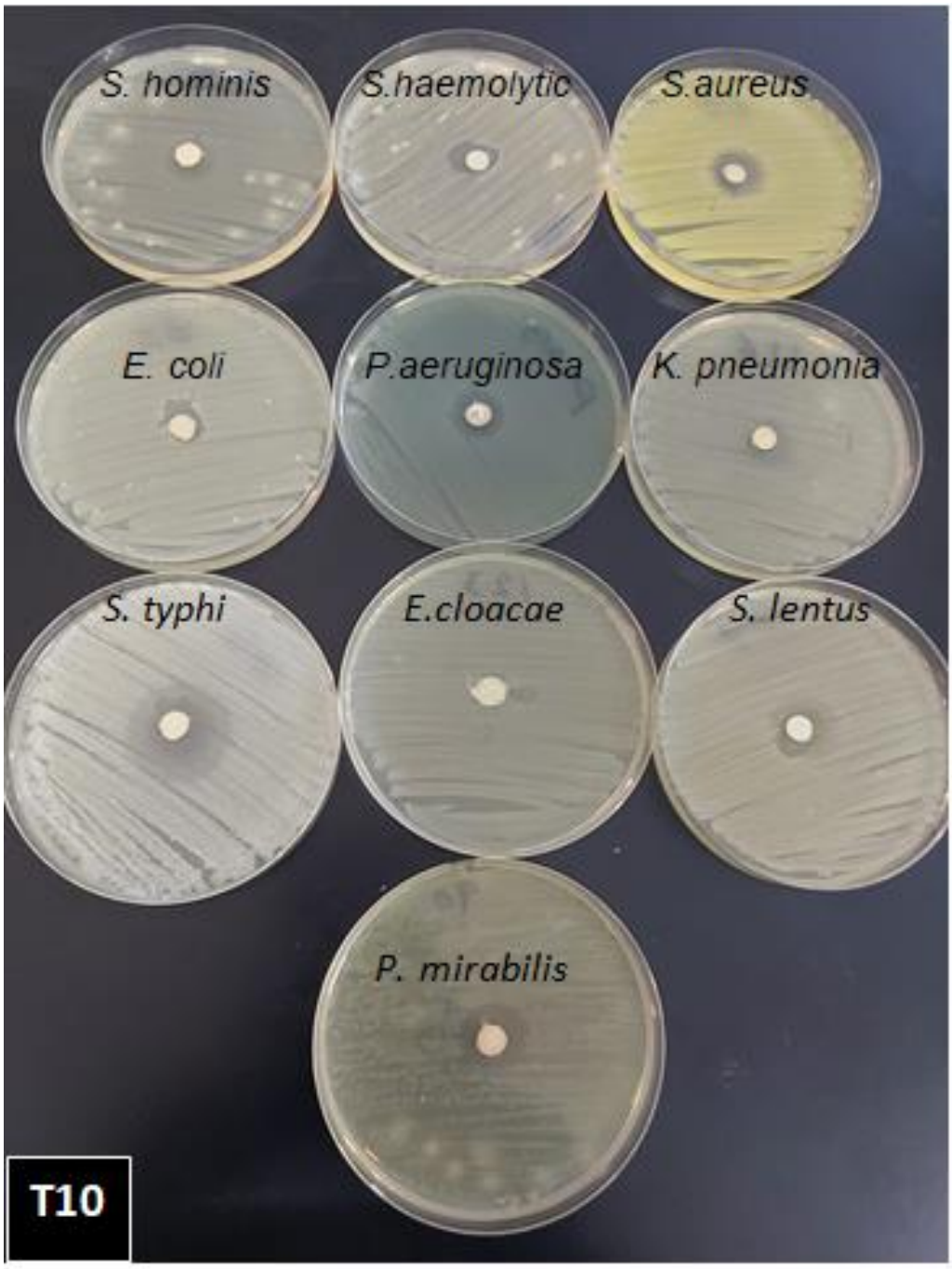


Figure3.16 Inhibition Zone on ten Pathogenic Bacteria by Biosynthesis AgNPs from *S.venezuelae.*

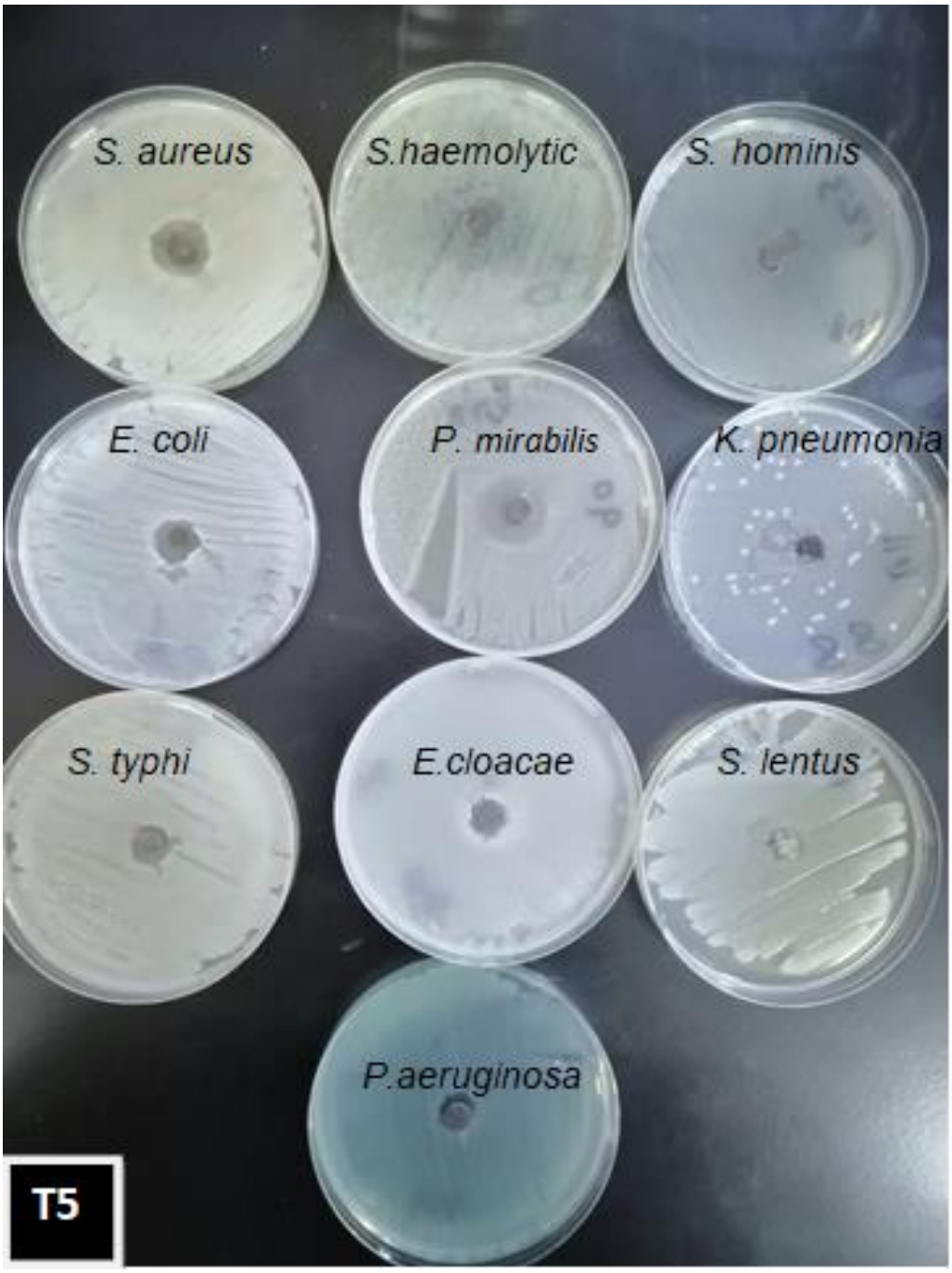


Figure3.17 Inhibition Zone on ten Pathogenic Bacteria by Biosynthesis AgNPs from *R.pusense.*

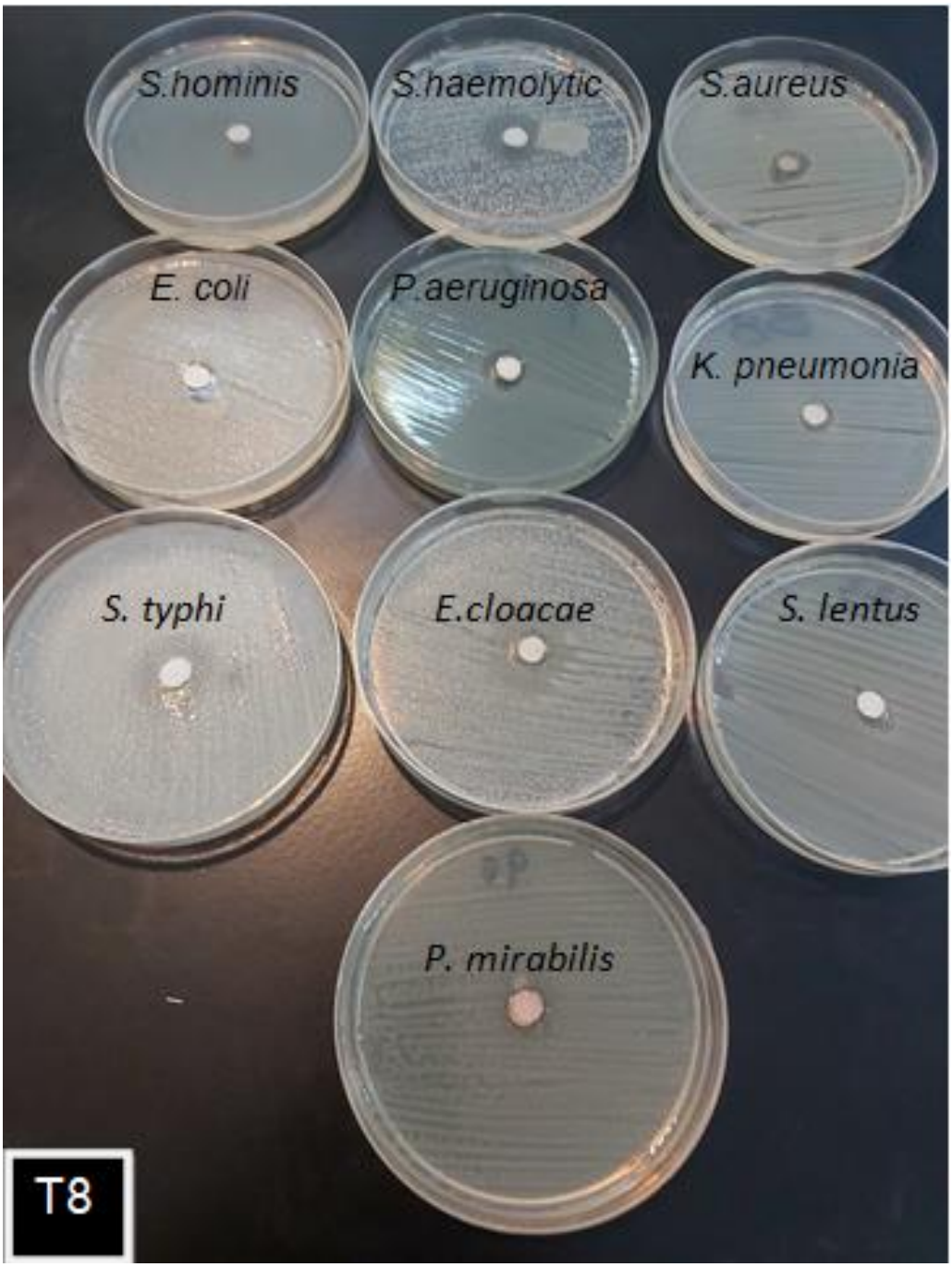


Figure 3.18 Inhibition Zone on ten Pathogenic Bacteria by Biosynthesis AgNPs from *C.respiraculi*

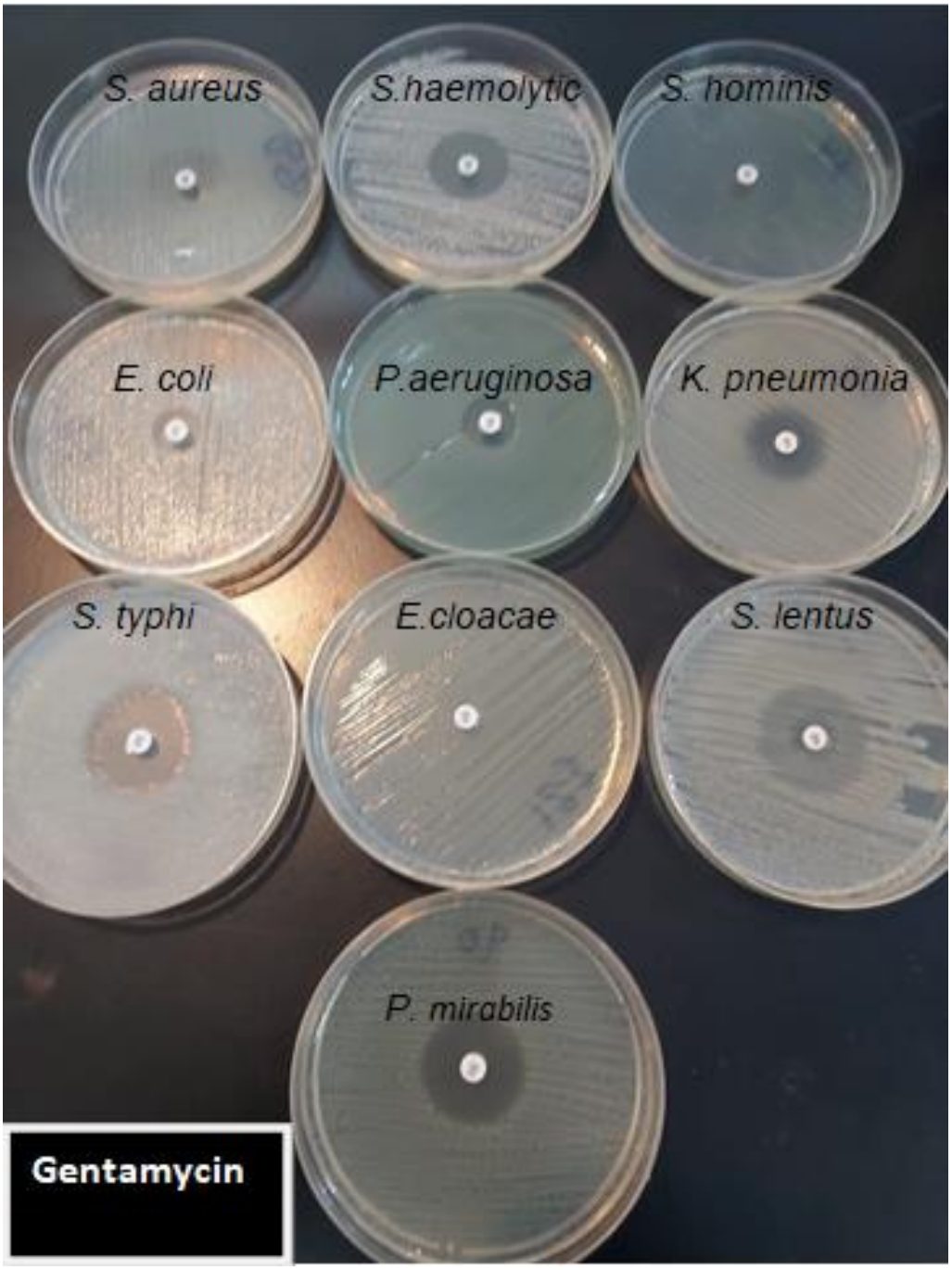


Figure 3.19 Inhibition Zone on ten Pathogenic Bacteria by antibiotic Gentamycin Antibiotic as a control.

Table 3.6: AgNPs Synthesized from Different Bacteria Isolated from Soil.

AgNPs	AgNPs Synthesized Bacteria	Strain
T1	*P. mosselii*	XG1-1-2
T2	*M.lacticm*	STM54
T3	*P.koreensis*	Allawy
T4	*R.pickettii*	Dewan 7
T5	*R.pusense*	WTB70
T6	*R.pickettii*	CHP10
T7	*R.solanceaum*	Mehbas
T8	*C.respiraculi*	Halema
T9	*P.putida*	LW
T10	*S.venezuelae*	JmKmYa 12

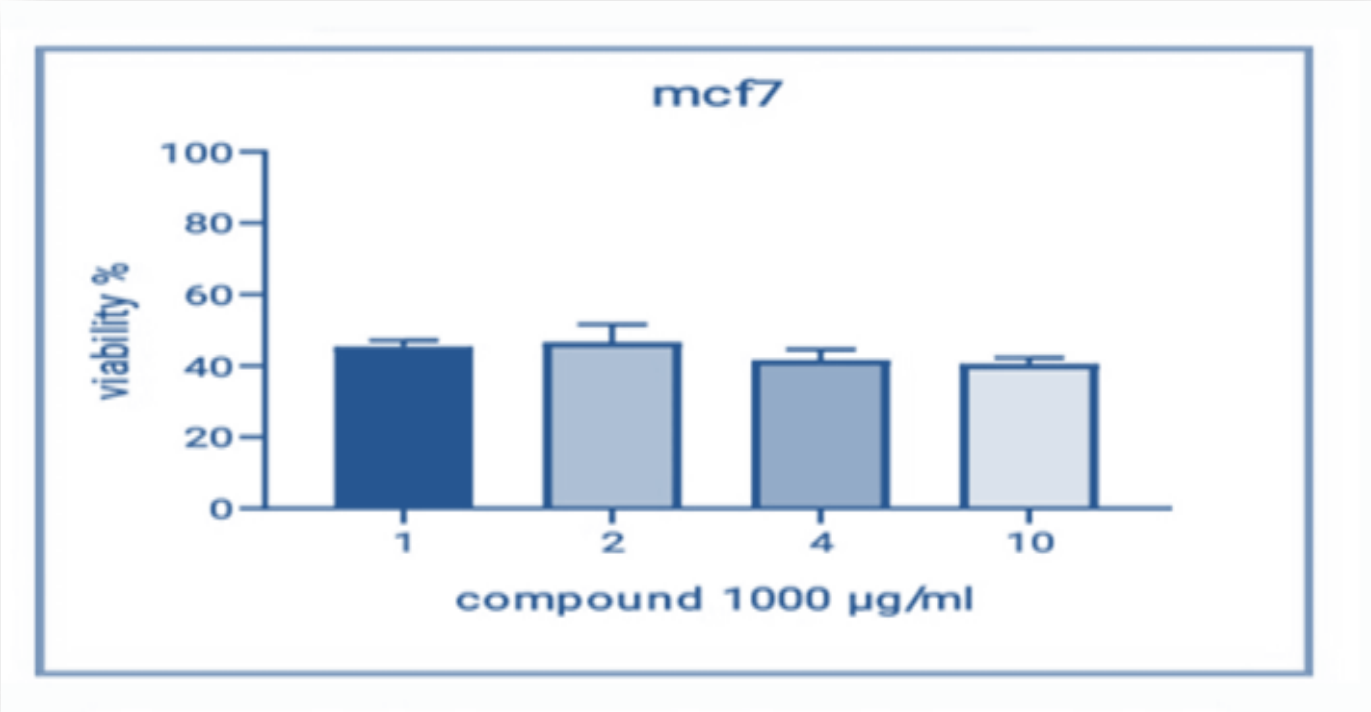
The AgNPs were testing for antimicrobial activity against pathogenic bacteria to determinate the inhibition zone .Compared with inhibition zone of the antibiotic (Gentamycin) as a control figure(3.19), we saw large inhibition zone on pathogenic bacteria by synthesized AgNPs , the largest inhibition zone was 49 mm on *S.hominis* by (T1) AgNPs, 47.3 mm on *S.aureus* by (T9) AgNPs, 23.5mm on *E. cloacae* by(T8) AgNPs, 40 mm on *P.aeruginosa* by (T7) AgNPs, 39.5 mm on *p.mirabilis* by (T3), (T1), AgNPs, 38.5mm on *P.aeruginosa* by (T4) AgNPs, 38mm on *S.aureus* by (T2) AgNPs,

35.5mm on*Salmonella typhi* by (T6) AgNPs, 27.5mm on *S. hominis* by (T5) AgNPs, 23 mm on *Salmonella typhi* by (T10) AgNPs. While the largest inhibition zone was 28mm on *S. lentus* by Gentamicin antibiotic. Largest inhibition zone of the synthesized AgNPs on pathogenic bacteria because nanoparticles affected on either the cell wall causes cell wall distraction or formation of reactive oxygen species (ROS) causes DNA, RNA, and proteins degrading or low production of adenosine triphosphate (ATP), all that reasons lead to cell death (Nguyen, V. T., *et.al*. 2019).

3.5.2.2 Antitumor Activity of Biosynthesized AgNPs on MCF7 Cell Line

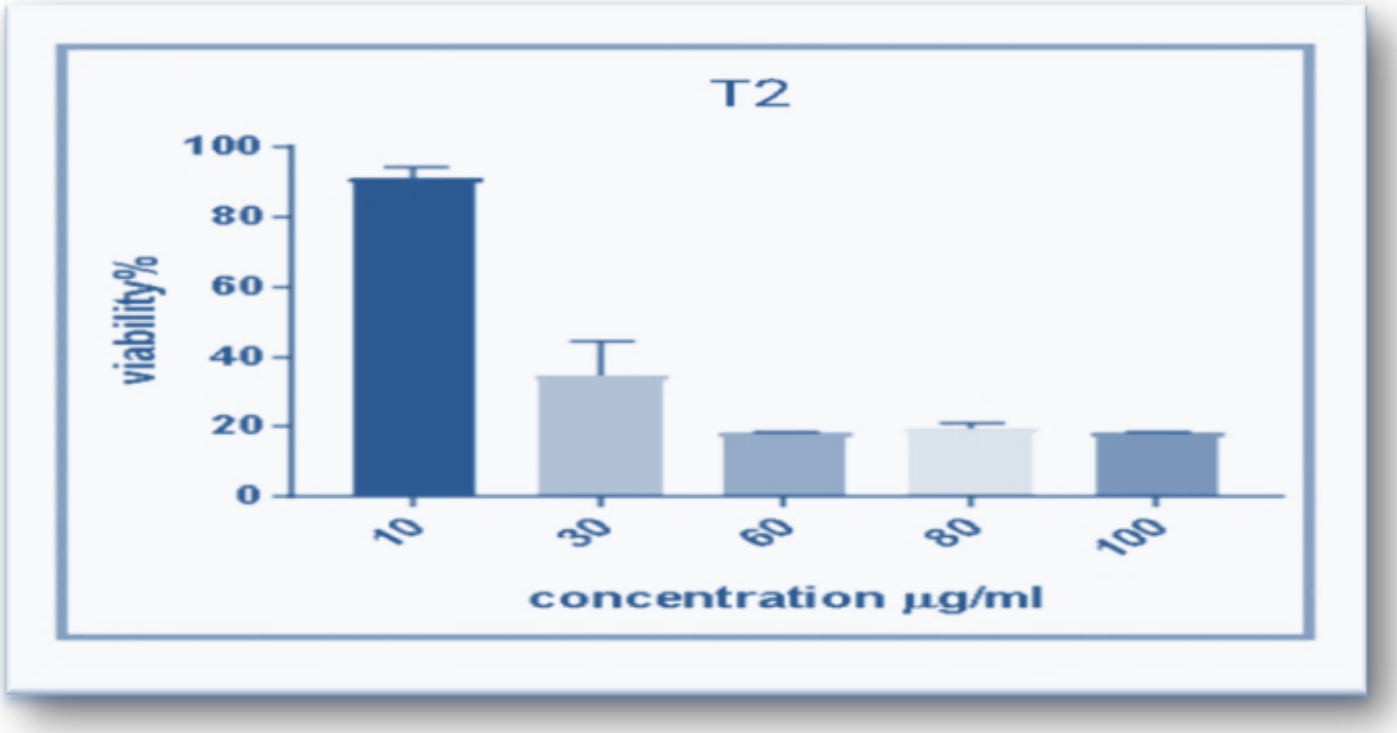
The anticancer activities of extracellular AgNPs from bacteria (selected 4samples)were determined using the MTT test against MCF7 (breast cancer cells), with a concentration of 1000 ug/ml in stage one to examine if there was any antitumor activity on MCF7.
After incubating the cell lines with the specified doses for 72 hours at 37 degrees Celsius, the results revealed that cell inhibition was for the 4 types of AgNPs (53.33089, 54.5205, 58.27233, 59.37042), indicating that the rate of inhibition is good as shown in figure (3.20). That lead us to the second stage of experiment.
The second stage

the AgNPs samples T2 and T10 were two of biosynthesized that were chosen to do the second stage. Multiple concentrations of chosen silver nanoparticles were added in this stage (100, 80, 60, 30, and 10µg/mL) for each sample. Following the incubation period, The optical densities of the cell lines with the indicated concentrations were measured in triplicates at 620nm at 37C for 72 hours. For the MCF7 cell line employed , the results shown in figure (3.21) and figure (3.23) summarize the mean percent cell viability with its standard deviation.



Compound 1mg/ml	1	2	4	10
Viability%	45.47	46.66	41.72	40.62

Figure 3.20 On the mcf7 Cell Line, the Rate of Viability percent for Each Sample is 1000µg/ml.



Compound µg/ml	10	30	60	80	100
Viability%	90.837	34.748	18.435	19.553	18.268

Figure 3.21: Compound T2 Synthesize by *M.lacticum* Bacteria Viability Percent on the MCF7 Cell Line.

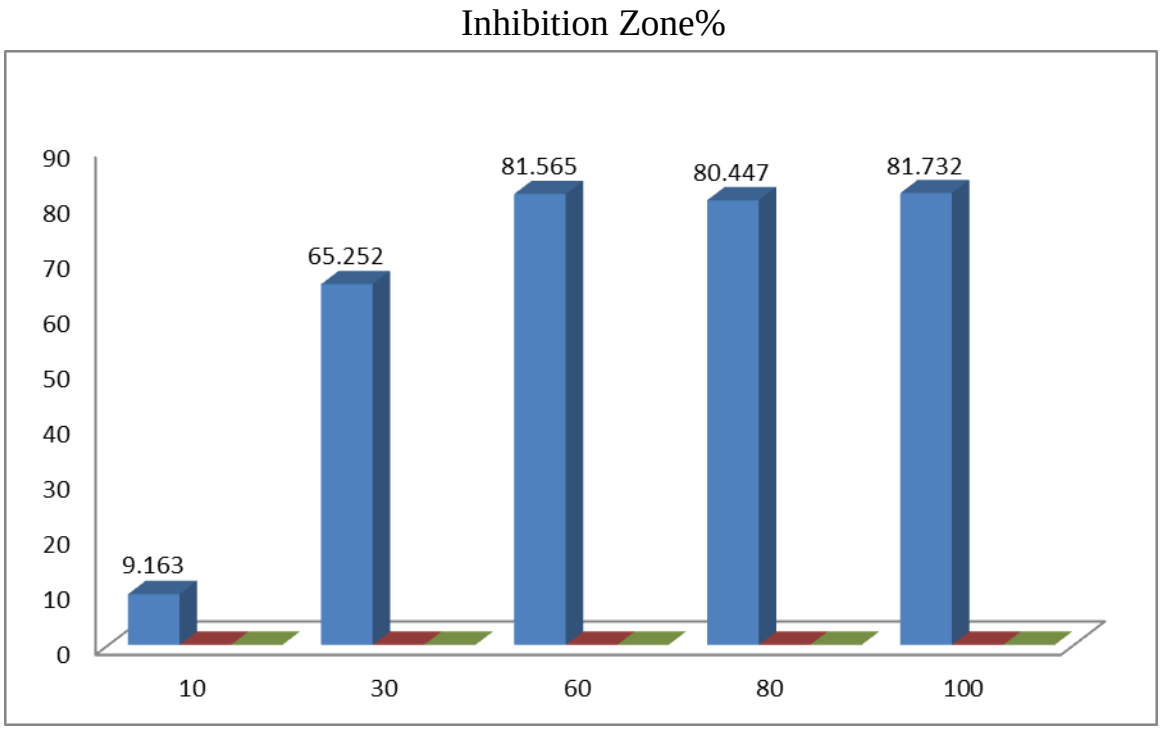
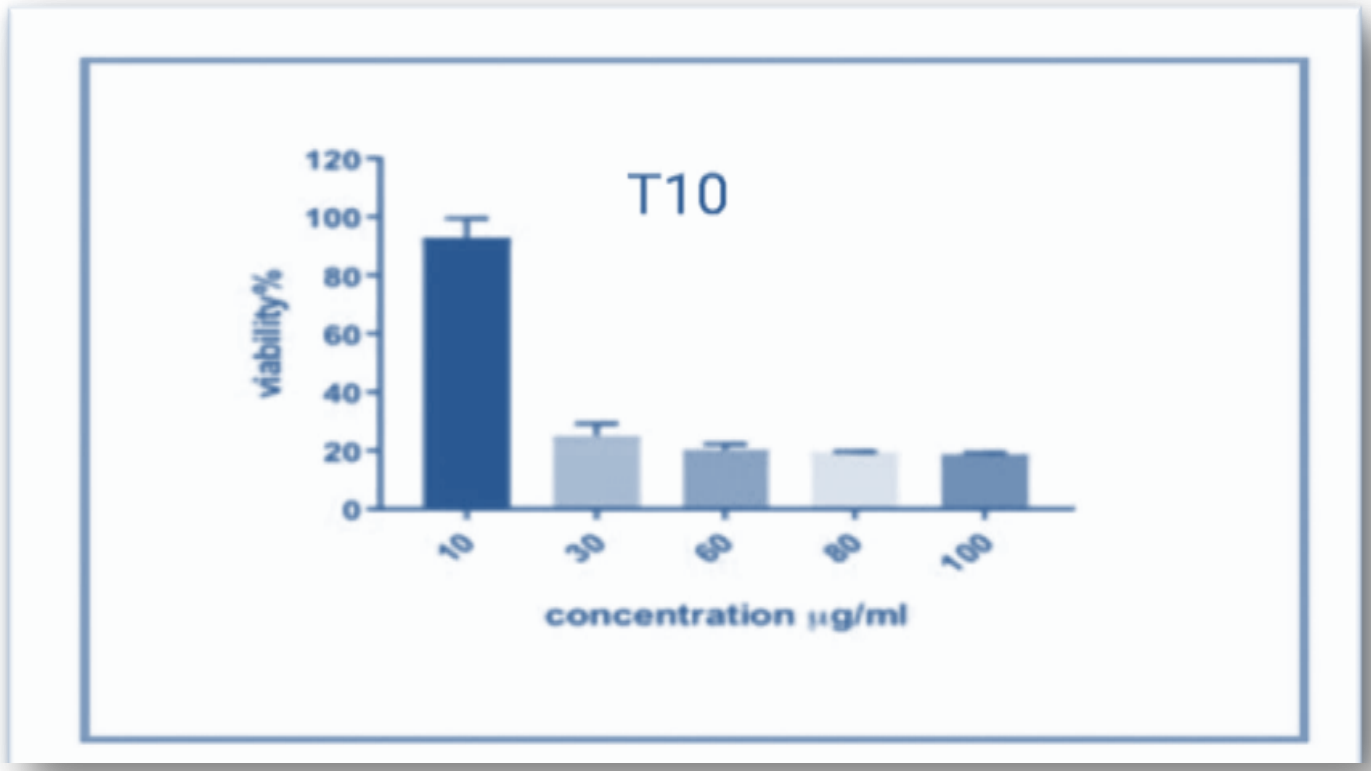


Figure 3.22 Inhibition Zone on MCF7 Cell Line by AgNPs(T2) Biosynthesize from *M.lacticum*.

After 72 hours of incubation, the antitumor activities of extracellular AgNPs on MCF7 (b reast cancer cells) showed the highest result (cell inhibition), with 100 µg/mL inhibiting 8 1.73 percent and 81.22 percent of MCF-7, respectively, implying that only 18.27 percent and 18.68 percent of MCF7 were able to form in soluble formazan products and remained as alive cells (viability rate was decreased which means that the rate of grown cancer cell lines will be inhibited so could not convert MTT compound and formation insoluble formazan products). The 10µg /ml concentration on the other hand, had the lowest outcome , inhibiting just 9.16 percent and 7.19 percent of breast cancer cells, respectively, as shown in figure (3.22) and figure (3.24). This indicates that as the concentration of AgNPs rises, so does its toxicity.



Compound µg/ml	10	30	60	80	100
Viability%	92.808	25.176	20.303	19.540	18.776

Figure 3.23: Compound T10 Synthssize by *S.venezuelae* Bacteria Viability Percent on the MCF7 Cell Line

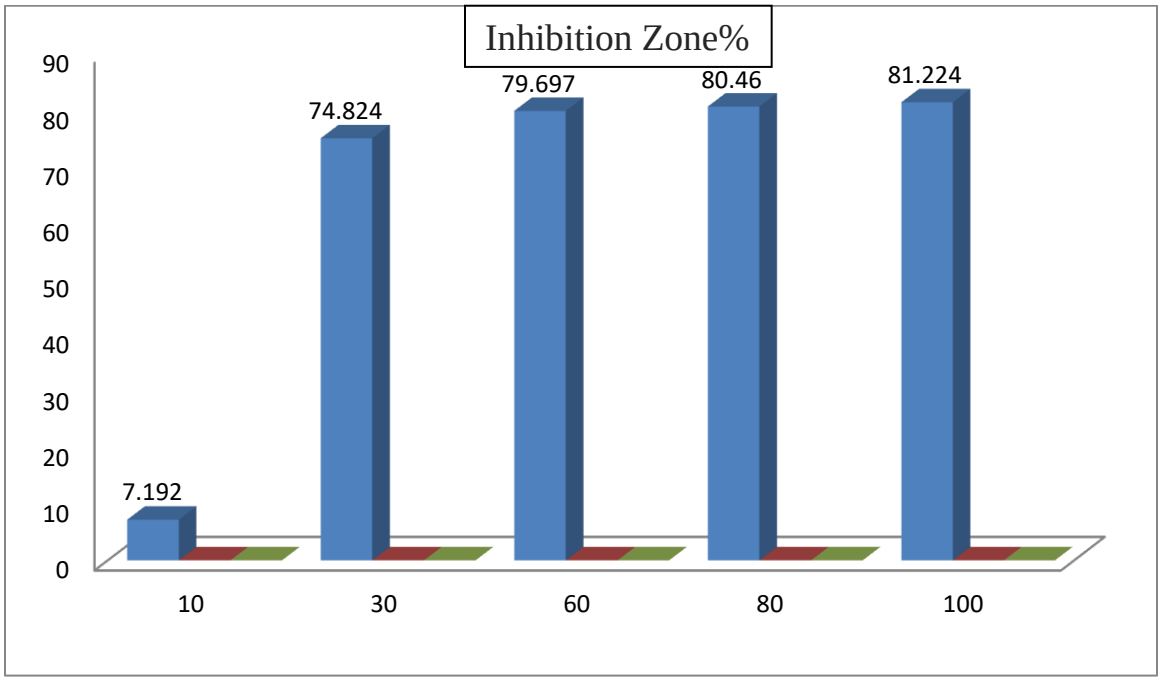


Figure 3.24: Inhibition Zone on MCF7 Cell Line by AgNPs(T10) Biosynthesize from *S.venezuelae* Bacteria.

The median inhibitory dose (IC50) value of 24.93µg/ml (sample T2) and 21.96µg /ml (sample T10). This means that biosynthesis AgNPs having excellent antitumor activity on breast cancer cells. The results showed that AgNPs strongly suppressed MCF-7 cell proliferation, This results agreed with those described by (Ravikumar *et al.* 2008), (Wadkins *et al.* 1998) and (Rashad *et al*. 2015), who found that the mechanism of action of some of these compounds isolated and extracted from some species of *Streptomyces* some genus of actinomycetes which cause harmful effects on fast multiplying cells by preventing DNA dependent RNA polymerase activities by intercalating with genetic materials, particularly duplex DNA. DNA cleavage is caused by inhibition of topoisomerase I or II. Inhibition of key enzymes, such as proteases,

mitochondrial permeabilization, and cellular metabolism, impairs signal transduction, as well as tumor-induced angiogenesis in some cases (Olano *et al*., 2009).

From all results the silver nanoparticles that were biosynthesized from *Microlacticum lacticum* shown very good results in all tested, as shown in table (3.7).

Table 3.7 The Results of (T2) AgNPs Synthesized from *Microlacticum lacticum*

Type of AgNPs	Color of Supernatant	UV-vis. Test	XRD Test	TEM Test	EDX Test	SEM Test	Antibacterial Test	Cytotoxicity Test
T2	Reddish Brown	489nm	Nanocrystal FCC(122), (111) and (200),	Scale 50 nm	Very Sharp Silver Beak	20-70nm	38mm	81.7%

CHAPTER FOUR

CONCLUSIONS & RECOMMENDATIONS

Conclusions

1-In the current study, Soil was shown to be a rich supply of various bacteria, including even novel strains were discovered from it during our work (seven new strains).

2-Actinomycetes and other isolated bacteria were used as extracellular biogenic AgNPs .

3- Elemental silver and its crystalline structure is confirmed by EDX, SEM, TEM, XRD and AFM investigation.

4-Both pathogenic Gram-negative and Gram-positive bacteria have been shown to be susceptible to AgNPs .

5- As Compared to commercially available antimicrobial drugs, the current study provides an environmentally friendly and cost-effective approach for the synthesis of powerful biologically produced silver nanoparticles against pathogenic bacteria, Which may used as alternative to traditional antibiotics.

6- AgNPs significantly suppressing MCF7 cell multiplication.

Recommendations

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

2.Using organisms other than bacteria biogenic AgNPs such as fungus, algae, and other organism.

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

4. Investigating a variety of physiochemical parameters, including investigation about their solubility, melting temperatures, zeta potential and other properties.

5.Study the direct effects of silver nanoparticles on bacteria by using liquid media to see which part of bacteria was affected by the AgNPs .

6.Toxicity tests by silver nanoparticles on experimental animals or on Red blood cells.

7.
We recommended performing activity tests for silver nanoparticles on some type of Dermatophytes .

8.
Using other cancer cells line to determinate the antitumor activity of AgNPs Such as PC3 and A375 cells line .

References

Abbaszadegan, A.; Ghahramani, Y.; Gholami, A.; Hemmateenejad , B.;Dorostkar, S.; Nabavizadeh, M. and Sharghi, H. (2015). The Effect of Charge at the Surface of Silver Nanoparticles on Antimicrobial Activity against Gram-Positive and Gram-Negative Bacteria. *J. Nanomaterials*, 3(4):1-8.

Abd-Elnaby, H. M., Abo-Elala, G. M., Abdel-Raouf, U. M., & Hamed, M. M. (2016). Antibacterial and anticancer activity of extracellular synthesized silver nanoparticles from marine Streptomyces rochei MHM13. *The Egyptian Journal of Aquatic Research, 42(3), 301-312.*

Abdulhassan,A.J. (2016). Effect of Silver and Titanium Nanoparticles Synthesized by Lactobacillus as Antimicrobial ,Antioxidant and Some Physiological Parameters .Master Thesis .University of Kufa ,Faculty of Science – Iraq. pp 86-93.

Ahmad, A.; Khan, M.I.; Kumar, R. (**2004).** Microbial Nanoparticle Production. Nanobiotechnology, Concepts, Applications and Perspectives; Niemeyer, C.M., Mirkin, C.A., Eds.; Wiley-VCH: Weinheim, Germany, pp. 126–135.

Ahmad, E., Sherwani, A., Owais, M., & Azam, A. (2013). Antibacterial and cytotoxic efficacy of extracellular silver nanoparticles bio-fabricated from chromium reducing novel OS4 strain of *Stenotrophomonas maltophilia*. PLoS One, 8(3), e59140. https://doi.org/ 10.1371/*journal .pone*. 0059140.

Alani F, Moo-Young M, Anderson W (2012). Biosynthesis of silver nanoparticles by a new strain of Streptomyces sp. compared with Aspergillus fumigatus. *World J Microbiol Biotechnol* 28(3):1081– 1086.

Al-Ali, A.A., Alsalami, K.A.S. and Athbi, A. M. (2022). Cytotoxic effects of CeO2 NPs and β- carotine and thier ability to induce apoptosis in human breast normal and cancer cell lines. *Iraqi Journal of Science,* V(63): 3.

Aldujaili, N. H., Alzubaidy, F. H., & Hussein, A. A. (2020). Extracellular synthesis of silver nanoparticles by *Acinetobacter baumanii* and antibacterial characterization. *Annals of Tropical Medicine and Public Health*, 23, 231-804.

Al Hannan, F., Keogh, M. B., Taha, S., & Al Buainain, L. (2019). Characterization of BRCA1 and BRCA2 genetic variants in a cohort of Bahraini breast cancer patients using next-generation sequencing. *Molecular genetics & genomic medicine, 7(7), e00771.*

Amin, S. M. M. (2016). Production, Purification, Characterization of Antibiotic from Locally Isolated of Actinomycetes spp. and Studying its Cytotoxicity and Antitumor Activity. (*Doctoral dissertation, Yarmouk University-Irbid-Jordan/2011).*

Anand, K.; Tiloke, C.; Naidoo, P.; Chuturgoon, A.A. (**2017).** Phytonanotherapy for management of diabetes using green synthesis nanoparticles. *J. Photochem. Photobiol. B, 173, 626–639. [CrossRef]*.

Antoniou, A., Pharoah, P. D., Narod, S., Risch, H. A., Eyfjord, J. E., Hopper, J. L., ... & Easton, D. (2003). Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case series unselected for family history: a combined analysis of 22 studies. *The American Journal of Human Genetics*, *72*(5), 1117-1130.

Balagurunathan R, Radhakrishnan M, Rajendran RB, Velmurugan D (2011). Biosynthesis of gold nanoparticles by actinomycete Streptomyces viridogens strain HM10. *J Biochem Biophys* 48: 331–335

Baranwal, A., Srivastava, A., Kumar, P., Bajpai, V. K., Maurya, P. K., & Chandra, P. (2018). Prospects of nanostructure materials and their composites as antimicrobial agents. *Frontiers in microbiology*, *9*, 422.

Bernard, B. (2007). Access excellence@ the national health museum. Isolation of antibiotic strains from soils.(www. Access excellence. Org.).

Bhosale, R. R.;Kulkarni, A.S.; Gilda, S.S.; Aloorkar, N.H.; Osmani R.A. and Harka B.R. (2014). Innovative Eco-friendly Approaches for Green Synthesis of Silver Nanoparticles. Int J Pharm Sci Nanotech, 7(1): 2328–2337.

Busi, S., Rajkumari, J., Ranjan, B., *et al.* (2014).'Green rapid biogenic synthesis of bioactive silver nanoparticles (AgNPs) using Pseudomonas aeruginosa', *IET Nanobiotechnol*., , 8, pp. 267–274 .

Chan-Yeung, M. N. (2000). Air pollution and health. Hong Kong Med J, 6(4), 390-398. Colvin, V. L. (2003). The potential environmental impact of engineered nanomaterials. *Nature Biotechnology*, 21(10), 1166-1170.

Chauhun R, Kumar A, Abraham J (2013). A biological approach to the synthesis of silver nanoparticles with Streptomyces sp JAR1 and its antimicrobial activity. Sci Pharm 81:607–621.

Clarridge III, J. E. (2004). Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. Clinical microbiology reviews, 17(4), 840-862.

Clinical and Laboratory Standards Institute (CLSI). (2018). Performance standards for antimicrobial susceptibility testing. Twenty-eight informational supplement M100-S25. Clinical and Laboratory Standards Institute, Wayne, PA.

Cooper, D.; Denneulin, T.; Bernier, N.; Béché, A. and Rouvière, J. L. (2016). Strain mapping of semiconductor specimens with nm-scale resolution in a transmission electron microscope. Micron, 80: 145– 165

Das, V. L., Thomas, R., Varghese, R. T., Soniya, E. V., Mathew, J., & Radhakrishnan, E. K. (2014). Extracellular synthesis of silver nanoparticles by the Bacillus strain CS 11 isolated from industrialized area. *3 Biotech*, *4*(2), 121-126.

Dayma, P. B., Mangrola, A. V, Suriyaraj, S. P., Dudhagara, P., & Rajesh, K. (2019). Synthesis of Bio-Silver Nanoparticles Using Desert Streptomyces Intermedius and Its Antimicrobial Activity Isolated. *Journal of Pharmaceutical, Chemical and Biological Sciences*, *7*(August), 94–101 .

Deepa S, Kanimozhi K, Panneerselvam A (2013). Antimicrobial activity of extracellularly synthesized silver nanoparticles from marine derived actinomycetes. Int *J Curr Microbiol Appl Sci* 2(9):223– 230

Devadass, B. J., Paulraj, M. G., Ignacimuthu, S., Theoder, P. A. S., & Dhabi, N. A. A. (2016). Antimicrobial activity of soil actinomycetes isolated from Western Ghats in Tamil Nadu, India. *J Bacteriol Mycol Open Access*, *3*(2), 224-232.

Duhan, J.S., Gahlawat, S.K. (2015). 'Biogenesis of nanoparticles: a review', *Afr. J. Biotechnol.* , 13, pp. 2778–2785.

Durán, N., Marcato, P.D., Duran, M., *et al*. (2011). 'Mechanistic aspects in the biogenic synthesis of extracellular metal nanoparticles by peptides, bacteria, fungi, and plants', *Appl. Microbiol. Biotechnol.*, 90, pp. 1609–1624.

Durán, N., Marcato, P. D., De Souza, G. I., Alves, O. L., & Esposito, E. (2007). Antibacterial effect of silver nanoparticles produced by fungal process on textile fabrics and their effluent treatment. *Journal of biomedical nanotechnology*, *3*(2), 203-208.

Elbeshehy, E. K., Elazzazy, A. M., & Aggelis, G. (2015). Silver nanoparticles synthesis mediated by new isolates of Bacillus spp.. *Frontiers in Microbiology*, 6, 453. https://doi.org/10.3389/fmicb.2015.00453.

El-Shanshoury, A.E.R.R., ElSilk, S.E., Ebeid, M.E. (2011).'Extracellular biosynthesis of silver nanoparticles using *Escherichia coli* ATCC 8739, *Bacillus subtilis* ATCC 6633, and *Streptococcus thermophilus* ESh1 and their antimicrobial activities', *ISRN Nanotechnol*., 2011, pp. 1–7.

Espinosa, J. C. M., Cerritos, R. C., Morales, M. A. R., Guerrero, K. P. S., Contreras, R. A. S., & Macías, J. H. (2020). Characterization of silver nanoparticles obtained by a green route and their evaluation in the bacterium of pseudomonas aeruginosa. *Crystals*, *10*(5), 1–13. https://doi.org/10.3390/cryst10050395 .

Feng, Q. L., Wu, J., Chen, G. Q., Cui, F. Z., Kim, T. N., & Kim, J. O. (2000). A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus. *Journal of biomedical materials research*, 52(4), 662-668.

Feynman, R. (2018). *There's plenty of room at the bottom* (pp. 63-76). CRC Press.

Fissan, H., Ristig, S., Kaminski, H., Asbach, C., Epple, M. (2014). Comparison of different characterization methods for nanoparticle dispersions before and after aerosolization. *Analytical Methods* 6: 7324-7334.

Forough, M. & Farhadi, K. (2010). Biological and green synthesis of silver noparticles. Turkish J. Eng. Env. Sci. 34 (2010) , 281 – 287.

Freshney, R.I. (2010). Culture of animal cells a manual of basic technique and specialized applications sixth edition, *Wiley-Blackwell*, P732.

Giovanna B. Giuseppantonio M. Semih E. (2016). Antimicrobial peptides and their interaction with biofilms of medically relevant bacteria. BBA – Biomembranes , 1858(5):1044-1060.

Govindaraju, K., Basha, S. K., Kumar, V. G., & Singaravelu, G. (2008). Silver, gold and bimetallic nanoparticles production using single-cell protein (Spirulina platensis) Geitler. *Journal of Materials Science*, *43*(15), 5115-5122.

Gould, J.C., Bowie, J.H. (1952). The determination of bacterial sensitivity to antibiotics. *Edinburgh medical journal* 59: 178.

Gullo, V. P.; McAlpine, J.; Lam, K. S.; Baker, D. and Petersen, F. (2006). Drug discovery from natural products. J Indust Microbiol Biotechnol. 33: 523–531.

Gupta, A. and Silver, S. (1998). Molecular Genetics: Silver as a biocide: Will resistance become a problem. *Nature Biotechnology*, 16: 888.

Haberland H. **(1994).** Clusters of Atoms and Molecules, Springer series in chemical physics, Berlin, New York.

Handy, R.D.; Henry, T.B.; Scown, T.M.; Johnston, B.D.; Tyler, C.R (2008). Manufactured nanoparticles: Their uptake and effects on fish-a mechanistic analysis. *Ecotoxicology*, 17, 396–409. [CrossRef].

Harley, J.P. and Prescott, L.M. (2002). Laboratory Exercises in Microbiology. 5th.ed. the McGraw-Hill Companies, Inc., New York.

Hassan,H, H, (2018). Biosynthesis and characterization of Ag Nanoparticles from *Klebsiella pneumoniae*.

Hallol, M. M. A. M. A. (2013). Studies on Bacterial Synthesis of Silver Nanoparticles Using Gamma Radiation and Their Activity against Some Pathogenic Microbes.

Helland, Å. (2004). Nanoparticles: a closer look at the risks to human health and the environment perceptions and precautionary measures of industry and regulatory bodies in Europe.

Huang, H. H., Ni, X. P., Loy, G. L., Chew, C. H., Tan, K. L., Loh, F. C., ... & Xu, G. Q. (1996). Photochemical formation of silver nanoparticles in poly (N-vinylpyrrolidone). *Langmuir*, *12*(4), 909-912.

Huq, M. (2020). Green Synthesis of Silver Nanoparticles Using Pseudoduganella eburnea MAHUQ-39 and Their Antimicrobial Mechanisms Investigation against Drug Resistant Human Pathogens. *International journal of molecular sciences, 21(4), 1510.*

Hochella, M.F (2016). Broadening nanotechnology's impact on development. Nat. Nanotechnol. 11, 398–400. [CrossRef] [PubMed] .

Hussain, S.M.; Hess, K.L.; Gearhart, J.M.; Geiss, K.T.; Schlager, J.J (2005) .

In vitro toxicity of nanoparticles in BRL 3A rat liver cells. Toxicol. Vitr. 19, 975–983. [CrossRef] .

H.Q.Yin, R.Langford, and R.E.Burrell, (1999). Comparative evaluation of the antimicrobial activity of ACTICOAT antimicrobial barrier dressing. *J. Burn. Care Rehabil. 20, 195.*

Ingle, A., Gade, A., Pierrat, S., Sonnichsen, C., & Rai, M. (2008). Mycosynthesis of silver nanoparticles using the fungus Fusarium acuminatum and its activity against some human pathogenic bacteria. *Current Nanoscience*, *4*(2), 141-144.

Ismaeel, H. M. (2013). Identification of genomic markers by RAPD–PCR Primers in Iraq Breast Cancer patients. *Iraq J Sci,* 54, 97-104.

Jain, D., Kachhwaha, S., Jain, R., *et al*. (**2010).** 'Novel microbial route to synthesize silver nanoparticles using spore crystal mixture of Bacillus thuringiensis', *Ind. J. Exp. Biol*., 48, p. 1152

Javaid, A., Oloketuyi, S. F., Khan, M. M., & Khan, F. (2018). Diversity of bacterial synthesis of silver nanoparticles. *BioNanoScience*, *8*(1), 43-59.

Jin, Y. (2020). Blood DNA methylation as a surrogate epigenetic biomarker in study of night shift work and breast cancer (Doctoral dissertation, Yale University).

Juibari, M.M., Abbasalizadeh, S., Jouzani, G.S., *et al* (2011). 'Intensified biosynthesis of silver nanoparticles using a native extremophilic Ureibacillus thermosphaericus strain', Mater. Lett., 65, pp. 1014–1017.

Kalabegishvili T, Kirkesali E, Ginturi E, Rcheulishvili A, Murusidze I, Pataraya D, Gurielidze M, Bagdavadze N, Kuchava N, Gvarjaladze D,

Lomidze L (2013). Synthesis of gold nanoparticles by new strains of thermophilic actinomycetes. Nano Stud 7:255–260

Karthik L, Kumar G, Vishnu Kirthi A, Rahuman AA, Bhaskara Rao KV (2014). Streptomyces sp. LK3 mediated synthesis of silver nanoparticles and its biomedical application. Bioprocess Biosyst Eng 37: 261–267.

Kasithevar, M., Saravanan, M., Prakash, P., Kumar, H., Ovais, M., Barabadi, H., & Shinwari, Z. K. (2017). Green synthesis of silver nanoparticles using Alysicarpus monilifer leaf extract and its antibacterial activity against MRSA and CoNS isolates in HIV patients. *Journal of Interdisciplinary Nanomedicine*, *2*(2), 131-141.

Khandel, P., & Kumar Shahi, S. (2016). Microbes mediated synthesis of metal nanoparticles: current status and future prospects. *International Journal of Nanomaterials and Biostructures*, *6*(1), 1–24.

Khawla IbrahimAlsamhary (2020), Eco-friendly synthesis of silver nanoparticles by *Bacillus subtilis* and their antibacterial activity.

Kim, J. S., Kuk, E., Yu, K. N., Kim, J. H., Park, S. J., Lee, H. J., ... & Cho, M. H. (2007). Antimicrobial effects of silver nanoparticles. *Nanomedicine: Nanotechnology, biology and medicine*, *3*(1), 95-101.

Klaus, T., Joerger, R., Olsson, E., & Granqvist, C.-G. (1999). Silver-based crystalline nanoparticles, microbially fabricated.Proceedings of the National Academy of Sciences of the United States of America, 96(24), 13611–13614.

Lazarovits, J., Chen, Y. Y., Sykes, E. A., & Chan, W. C. (2015). Nanoparticle–blood interactions: the implications on solid tumour targeting. *Chemical Communications*, *51*(14), 2756-2767.

Liau, S. Y., Read, D. C., Pugh, W. J., Furr, J. R., & Russell, A. D. (1997). Interaction of silver nitrate with readily identifiable groups: relationship to the antibacterialaction of silver ions. *Letters in applied microbiology*, *25*(4), 279-283.

Lieber, C.M.; Gao, R.; Strehle, S (2017) . Nanoscale Sensors for Intracellular and Other Applications. *American Patent* No. U.S. 9638717 B2, 2 May.

Li, Q.; Mahendra, S.; Lyon, D.Y.; Brunet, L.; Liga, M.V.; Li, D.; Alvarez, P.J (2008) . Antimicrobial nanomaterials for water disinfection and microbial control: Potential applications and implications. *Water Res.*, 42, 4591–4602. [CrossRef] .

Manivasagan P, Venkatesan J, Senthilkumar K, Sivakumar K, Kim S (2013). Biosynthesis, antimicrobial and cytotoxic effect of silver nanoparticles using a novel Nocardiopsis sp. MBRC-1. *Bio Med Res Int* 2013 Article ID 287638 (9 pages).

Malmsten, M. (2014). Nanomaterials as antimicrobial agents. In *Handbook of nanomaterials properties* (pp. 1053-1075). *Springer, Berlin, Heidelberg*.

Mohammed, Z. A. M. (2011). Determination of Serum IL-8 Level in Women with Breast Cancer and Their Correlation With Disease Progression. *Iraqi Journal of Cancer and Medical Genetics*, *4*(2), 43-46.

Mohanpuria P, Rana KN, Yadav SK (2008). Biosynthesis of nanoparticles: technological concepts and future a p p l i c a t i o n s . *J N a n o p a r t . R e s .* 10: 507–517.

Murugan, K., Senthilkumar, B., Senbagam, D., & Al-Sohaibani, S.(2014). Biosynthesis of silver nanoparticles using Acacia leucophloea extract and their

antibacterial activity. *International Journal of Nanomedicin*, 9, 2431–2438. https://doi.org/10.2147/IJN.S61779.

M.Schaller, J.Laude, H.Bodewaldt, G.Hamm, and H.C.Korting, (2004). Toxicity and antimicrobial activity of a hydrocolloid dressing containing silver particles in an ex vivo model of cutaneous infection. *Skin Pharmacol. Physiol. 17, 31.*

Nayaka, S., Chakraborty, B., Bhat, M. P., Nagaraja, S. K., Airodagi, D., Swamy, P. S., ... & Kanakannanavar, B. (2020). Biosynthesis, characterization, and in vitro assessment on cytotoxicity of actinomycete-synthesized silver nanoparticles on Allium cepa root tip cells. *Beni-Suef University Journal of Basic and Applied Sciences*, *9*(1), 1-18.

Nanda, A., & Saravanan, M. (2009). Biosynthesis of silver nanoparticles from *Staphylococcus aureus* and its antimicrobial activity against MRSA and MRSE. *Nanomedicine: Nanotechnology, Biology and Medicine*, *5*(4), 452-456.

Naito M, Yokoyama T, Hosokawa K, Nogi K, editors (2018). Nanoparticle Technology Handbook. *Amsterdam: Elsevier*.

Narasimha G, Janardhan A, Alzohairy M, Khadri H,MallikarjunaK(2013) . Extracellular synthesis, characterization and antibacterial activity of silver nan oparticles by actinomycetes isolative. *Int J Nano Dimens* 4:77–83

Nallamuthu, T., Venkatalakshmi, R. P., Chinnasamy, A., & Kannaiyan, P.

(2012). Synthesis of silver nanoparticles and the antibacterial and anticancer activities of the crude extract of sargassum polycystum C. Agardh. *Nano Biomedicine and Engineering*, *4*(2), 89–94. https://doi.org/10.5101/nbe.v4i2.p89-94.

Newman, D. J. and Cragg, G. M. (2007). Natural products as sources of new drugs over the last 25 years. J Nat Prod. 70: 461−477.

Nguyen, V. T., Vu, V. T., Nguyen, T. A., Tran, V. K., & Nguyen-Tri, P. (2019). Antibacterial activity of TiO2-and ZnO-decorated with silver nanoparticles. *Journal of Composites Science*, *3*(2), 61.

Ogunsona, E. O., Muthuraj, R., Ojogbo, E., Valerio, O., & Mekonnen, T. H. (2020). Engineered nanomaterials for antimicrobial applications: A review. *Applied Materials Today*, *18*, 100473.

Olano, C.; Méndez, C. and Salas, J. A. (2009). Antitumor compounds from actinomycetes: from gene clusters to new derivatives by combinatorial biosynthesis. *Nat Prod Rep*. 26:628-660.

Otari, S. V., Patil, R. M., Ghosh, S. J., Thorat, N. D., & Pawar, S.H (2014). Intracellular synthesis of silver nanoparticle byactinobacteria and its antimicrobial activity. Spectrochimica Acta. Part A, Molecular and Biomolecular Spectroscopy, 136(PtB), 1175–1180. https://doi.org/10.1016/j.saa.2014.10.003.

Otari SV, Patil RM, Nadaf NH, Ghosh SJ, Pawar SH (2012). Green biosynthesis of silver nanoparticles from an actinobacteria Rhodococcus sp. Mater Lett 72:92–94.

Othman, A. M., Elsayed, M. A., Al-Balakocy, N. G., Hassan, M. M., & Elshafei, A. M. (2019). Biosynthesis and characterization of silver nanoparticles induced by fungal proteins and its application in different biological activities. *Journal of Genetic Engineering and Biotechnology, 17(1), 8.*

Oves, M., Khan, M. S., Zaidi, A., Ahmed, A. S., Ahmed, F., Ahmad, E., ... & Azam, A. (2013). Antibacterial and cytotoxic efficacy of extracellular silver nanoparticles biofabricated from chromium reducing novel OS4 strain of Stenotrophomonas maltophilia. PloS one, 8(3), e59140.

Oza G, Pandey S, Gupta A, Kesarkar R, Sharon M (2012). Biosynthetic reduction of gold ions to gold nanoparticles by Nocardia farcinica. *J Microbiol Biotechnol Res 4:511–515.*

Palmqvist, Martin (2017). Nanoparticles. Diss. (sammanfattning / summary) Uppsala : Sveriges lantbruksuniv., Acta Universitatis agriculturae Sueciae, 2017:105: 1652-6880.

Parashar, U.K.; Saxena, S.P.; Srivastava, A (2009). Bioinspired synthesis of silver nanoparticles. *Dig. J. Nanomat. Biostruct.*, 4, 159–166.

Pattekari, P., Zheng, Z., Zhang, X., Levchenko, T., Torchilin, V., & Lvov, Y. (2011). Top-down and bottom-up approaches in production of aqueous nanocolloids of low solubility drug paclitaxel. Physical Chemistry Chemical Physics, 13(19), 9014-9019.

Paumier, G. (2008). Ecole doctorale : G´enie ´electrique, ´electronique, t´el´ecommunications ´ Sp´ecialit´e : Conception des circuits micro´electroniques et microsyst`emes. PhD thesis. Pp:15.

Pharm, M. (2008). *Isolation and identification of Streptomyces species from Bangladeshi soil and studies on their secondary metabolites* (Doctoral dissertation, Doctoral dissertation, University of Rajshahi, Rajshahi, Bangladesh).

Pitkethy, M. J. (2003). Nanoparticles as building blocks?. *Materials Today*, *6*(12), 36-42.

Prakasham RS, Buddana SK, Yannam SK, Guntuku GS (2012). Characterization of silver nanoparticles synthesized by using marine isolate Streptomyces albidoflavus. *J Microbiol Biotechnol* 22:614– 621.

Prabhu, S. and Poulose, E. K. (2012): Silver nanoparticles: mechanism of antimicrobial action, synthesis, 109 medical applications, and toxicity effects. *International Nano Letters*, 2:32 .

Prathna, T. C., Chandrasekaran, N., & Mukherjee, A. (2011). Studies on aggregation behaviour of silver nanoparticles in aqueous matrices: effect of surface functionalization and matrix composition. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 390(1- 3), 216-224.

Rai, M.K.; Deshmukh, S.D.; Ingle, A.P. and Gade, A.K. (2012). Silver nanoparticles: The powerful nanoweapon against multidrug-resistant bacteria. *J. Appl. Microbiol.*; 112: 841–852.

Rai, M., Yadav, A., & Gade, A. (2009). Silver nanoparticles as a new generation of antimicrobials. *Biotechnology advances*, *27*(1), 76-83.

Rajora, N., Kaushik, S., Jyoti, A., & Kothari, S. L. (2016). Rapid synthesis of silver nanoparticles by *Pseudomonas stutzeri* isolated from textile soil under

optimised conditions and evaluation of their antimicrobial and cytotoxicity properties. *IET nanobiotechnology*, *10*(6), 367-373.

Rajamanickam U, Mylsamy P, Viswanathan S, Muthusamy P (2012). Biosynthesis of zinc nanoparticles using actinomycetes for antibacterial food packaging. International Conference on Nutrition and Food Sciences IPCBEE vol 39 IACSIT.

Ravikumar, Y. S.; Mahadevan, K. M.; Kumaraswamy, M. N.; Vaidya, V. P.; Manjunatha, H.; Kumar, V.(2008). Antioxidant, Cytotoxic and Genotoxic evaluation of alcoholic extract of Polyalthia cerasoides (roxb) Bedd. Environ Toxicol Pharmacol. 26: 142-146.

Rashad, F. M.; Nadia H A.; Insaf, E. D. and Fatima, H. M. (2015). Isolation and Characterization of Antibiotic / Antitumor Producing Streptomyces. RJPBCS. 6(2) :1917.

Reidy, B., Haase, A., Luch, A., Dawson, K. A., & Lynch, I. (2013). Mechanisms of silver nanoparticle release, transformation and toxicity: A critical review of current knowledge and recommendations for future studies and applications. *Materials*, *6*(6), 2295–2350. https://doi.org/10.3390/ma6062295.

Sadhasivam S, Shanmugam P, Yun K (2010). Biosynthesis of silver nanoparticles by Streptomyces hygroscopicus and antimicrobial activity against medically important pathogenic microorganisms. Colloids Surf B: Biointerfaces 81:358–362.

Sajid, M.; Llyas, M.; Basheer C (2015). Impact of nanoparticles on human and

environment: Review of toxicity factors, exposures, control strategies, and future prospects. Environ. Sci. Pollut. Res. Int., 22, 4122–4143. [CrossRef].

Sambrook, J., & Russell, D. W. (2006). Purification of nucleic acids by extraction with phenol: chloroform. Cold Spring Harbor Protocols, 2006(1), pdb-prot4455.

Sangour, M. H., Ali, I. M., Atwan, Z. W., & Al Ali, A. A. A. L. A. (2021). Effect of Ag nanoparticles on viability of MCF-7 and Vero cell lines and gene expression of apoptotic genes. Egyptian Journal of Medical Human Genetics, 22(1), 1-11.

Sanjenbam P, Gopal JV, Kannabiran K (2014). Anticandidal activity of silver nanoparticles synthesized using Streptomyces sp. VITPK1. J De Mycologie Médicale, Available from: http://dx.doi.org/10.1016/ *j.mycmed*.2014.03.004.

Sastry M, Ahmed A, Khan MI, Kumar R (2003). Biosynthesis of metal nanoparticles using fungi and actinomycetes. Curr Nanosci 85(2): 162–170.

Selvakumar P, Viveka S, Prakash S, Jasminebeaula S, Uloganathan R (2012). Antimicrobial activity of extracellularly synthesized silver nanoparticles from marine derived Streptomyces rochei. *Int J Pharm Biol Sci 3:188–197.*

Sharma, R.; Bisen, D. P.; Shukla, U. and Sharma, B. G. (2012). X-ray diffraction: a powerful method of characterizing nanomaterials. Recent Research in Science and Technology, 4(8), 77–79.

Shenashen, M.A.; El-Safty, S.A.; Elshehy, E.A.(2014). Synthesis, morphological control, and properties of silver nanoparticles in potential applications. Part. Part. Syst. Charact., 31, 293–316. [CrossRef].

Smuleac, V.; Xiao, L. and Bhattacharyya, D.(2013). Greener and Other Approaches To Synthesize Fe and Pd Nanoparticles in Functionalized Membranes and Hydrogel, ACS Symposium Series, Vol. 1124, Chapter 3, pp 41–58.

Silverstein, F. E., Faich, G., Goldstein, J. L., Simon, L. S., Pincus, T., Whelton, A., ... & Geis, G. S. (2000). Gastrointestinal toxicity with celecoxib vs nonsteroidal anti-inflammatory drugs for osteoarthritis and rheumatoid arthritis: the CLASS study: a randomized controlled trial. Jama, 284(10), 1247-1255.

Singh, M.; Singh, S.; Prasad, S. and Gambhir, I. S. (2008): Nanotechnology in Medicine and Antibacterial Effect of Silver Nanoparticles. *Digest Journal ofNanomaterials and Biostructures. 3(3): 115 – 122.*

Singh, O. V. (Ed.). (2015). Bio-nanoparticles: biosynthesis and sustainable biotechnological implications. John Wiley & Sons.

Soltaninezhad, M., Khatami, M., & SHAHIDI, B. G. (2015). *Streptomyces somaliensis* mediated green synthesis of silver nanoparticles.

Soltaninezhad, M., Khatami, M., & SHAHIDI, B. G. (2015). Streptomyces somaliensis mediated green synthesis of silver nanoparticles.

Staggers, N.; Mckasky, T.; Braselton, N.; Kennedy, R. (**2008).** Nanotechnology: The coming revolution and its implications for consumers, clinicians, and informatics. Nurs. Outlook, 56, 268–274. [CrossRef] [PubMed] .

Subashini J, Kannabiran K (2013). Antimicrobial activity of Streptomyces sp. VITBT7 and its synthesized silver nanoparticles against medically important fungal and bacterial pathogens. *Der Pharm Lett* 5: 192–200.

Sukanya MK, Saju KA, Praseetha PK, Sakthivel G (2013). Potential of biologically reduced silver nanoparticles from Actinomycete cultures. *J Nanosci 1-8.*

Sundarmoorthi E, Devarasu S, Vengadesh Prabhu K (2011). Antimicrobial and wound healing activity of silver nanoparticles synthesized from Streptomyces aureofaciens. *Int J Pharm Res Dev* 12:69–75.

Sunitha A, Rimal IRS, Geo S, Sornalekshmi S, Rose A, Praseetha PK (2013). Evaluation of antimicrobial activity of biosynthesized iron and silver nanoparticles using the fungi Fusarium oxysporum and Actinomycetes sp. on human pathogens. Nano Biomed Eng 5: 39- 45.

Sunitha A, Geo S, Sukanya S, Praseetha PK, Dhanya RP (2014). Biosynthesis of silver nanoparticles from actinomycetes for therapeutic applications. *Int J Nano Dimens 5:155–162.*

Sunkar, S., Nachiyar, C.V. (2012). 'Biogenesis of antibacterial silver nanoparticles using the endophytic bacterium Bacillus cereus isolated from Garcinia xanthochymus', *Asian Pac. J. Trop. Biomed.*, , 2, pp. 953–959 .

Taglietti, A.; Diaz Fernandez, YA. ; Amato, E. ; Cucca, L. ; Dacarro, G.; Grisoli, P.; Necchi, V. ; Pallavicini, P.; Pasotti, L. and Patrini, M. (2012). Antibacterial Activity of Glutathione-Coated Silver Nanoparticles against Gram Positive and Gram Negative Bacteria. Langmuir, 28 (21):8140–8148.

Thangaraju, N., Venkatalakshmi, R. P., Chinnasamy, A., & Kannaiyan, P. (2012). Synthesis of silver nanoparticles and the antibacterial and anticancer activities of the crude extract of Sargassum polycystum C. *Agardh. Nano Biomed Eng*, *4*(2), 89-94.

Thapa, R., Bhagat, C., Shrestha, P., Awal, S., & Dudhagara, P. **(2017).** Enzyme-mediated formulation of stable elliptical silver nanoparticles tested against clinical pathogens and MDR bacteria and development of antimicrobial surgical thread. Annals of clinical microbiology and antimicrobials, 16(1), 1-10.

Tran, S., DeGiovanni, P. J., Piel, B., & Rai, P. (2017). Cancer nanomedicine: a review of recent success in drug delivery. Clinical and translational medicine, 6(1), 1-21.

Tsibakhashvili NY, Kirkesali EI, Pataraya DT, Gurielidze MA, Kalabegishvili TL, Gvarjaladze DN, Tsertsvadze GI, Frontasyeva MV, Zinicovscaia II, Wakstein MS, Khakhanov SN, Shvindina NV, Shklover VY (2011). Microbial synthesis of silver nanoparticles by Streptomyces glaucus and Spirulina platensis. *Adv Sci Lett* 4:3408– 3417.

Usha R, Prabu E, Palaniswamy M, Venil CK, Rajendran R (2010). Synthesis of metal oxide nanoparticles by Streptomyces sp for development of antimicrobial textiles. *Glob J Biotechnol Biochem* 5:153–160.

Verma, P., & Maheshwari, S. K. (2018). Applications of Silver nanoparticles in diverse sectors. *International Journal of Nano Dimension*, *0*(0), 18–36.

Vengadesh Prabu K, Sundaramoorthi C, Devarasu S (2011). Biosynthesis of silver nanoparticles from Streptomyces aureofaciens. *J Pharm Res* 4:820–822.

Vidyasagar GM, Shankaravva B, Begum R, Imrose, Raibagkar RL (2012). Antimicrobial activity of silver nanoparticles synthesized by Streptomyces species JF714876. *Int J Pharm Sci Nanotechnol* 5:1638–1642.

Vinelli, A.; Primiceri, E.; Brucale, M.; Zuccheri, G.; Rinaldi, R. and Samorì, B. (2008). Sample preparation for the quick sizing of metal nanoparticles by atomic force microscopy. *Microsc. Res. Tech*. 2008, 71, 870−879.

Wadkins, R.M.;, Vladu, B. and Tung, C.S.(1998). Actinomycin D binds to metastable hairpins in single-stranded DNA. Biochem., 37(34):11915–1923.

Waghmare SS, Deshmukh AM, Kulkarni W, Oswaldo LA (2011). Biosynthesis and characterization of manganese and zinc nanoparticles. *Univ J Environ Res Technol 1:64–69.*

Waghmare SS, Deshmukh AM, Sadowski Z (2014). Biosynthesis, optimization, purification and characterization of gold nanoparticles. *Afr J Microbiol Res 8:138–146.*

Wei, Q.S., Fu, J.J.J. and Shen, J.C. (2007). Norvancomycin-capped silver nanoparticles: synthesis and antibacterial activities against *E. coli*. Science in China, Series B: Chemistry, 50, pp: 418– 424.

Williams D.B.and Carter, C.B. (2009). Transmission electron microscopy. Springer.The transmission electron microscope.3–22 better spatial resolution and capabil xylopyrus bark extract. *Appl Nanosci.*, 5:755–762.

Yang, **C. C., & Mai, Y. W. (2014**). Thermodynamics at the nanoscale: A new approach to the investigation of unique physicochemical properties of nanomaterials. Materials Science and Engineering: R: Reports, 79: 1-40.

Zhanga, Y., Chenga, X., Zhanga, Y., *et al*. (**2013).** 'Biosynthesis of silver nanoparticles at room temperature using aqueous aloe leaf extract and antibacterial properties', Colloid Surf. Physicochem. Eng. Aspects, , 423, pp.

63–68 .

Zhang, **X.-F.; Liu, Z.-G.; Shen, W. and Gurunathan, S. (2016).** Silver Nanoparticles: Synthesis, Characterization, Properties, Applications, and Therapeutic Approaches. *Inte. J. Mole. Sci.,* 17(9): 1534.

Zinjarde, S. S. (2012). Bio-inspired nanomaterials and their applications as antimicrobial agents. *Chronicles of Young Scientists*, *3*(1).

Zonooz NF, Salouti M (2011). Extracellular biosynthesis of silver nanoparticles using cell filtrate of Streptomyces sp. ERI-3. *Scientia Iranica* 18:1631–1635.

الخلاصه

تعد التكنولوجيا الحيوية النانوية مجالًا جديدًا ظهر نتيجة دمج تقنية النانو مع التكنولوجيا الحيوية. تتمتع التقنيات الحيوية النانوية بميزه عن الطرق غير البيولوجية. نتيجة لذلك ، كان الهدف من هذا البحث هو اختيار وتحسين السلالات المحتملة للتخليق الحيوي خارج الخلية لجسيمات الفضة النانويه ودراسة فعاليتها كمضاد بكتيري ومضاد لخلايا سرطان الثدي.

بداية العمل كانت بجمع عينات التربه من حقول السكرفي ميسان والحدائق من الفتره (24/11/2020 ولغاية 24/5/ 2021). ونقلت الى مختبرعلوم الحياة في كلية العلوم جامعة ميسان ليتم عزل البكتيريا هناك . تم أستخدام التشخيص الجيني للسلالات البكتيريه المعزوله من التربه عن طريق مقارنتها ببنك الجينات في كوريا وكذلك المركز الوطني لمعلومات التكنولوجيا الحيويه . ووجد أن بعض هذه السلالات كانت جديده وقد تم تسجيلها في بنك الجينات . ثم أستخدمت لغرض التصنيع الحيوي للجسيمات النانويه مختبريا وتم استخدام افضل الظروف من درجة حراره وأس هايدروجيني للتخليق الحيوي بعد فترة الحضانه تمت ملاحظة التغير اللوني للخليط إلى البني أو البني المحمر حسب نوع السلاله المنتجه للنانو وهو أول علامه على تصنيع الجسيمات النانويه نتيجه لعملية الأختزال الحيوي بواسطة أنزيم الريدكتيز الذي أفرزته البكتيريا المصنعه في الوسط بعد ذلك تم فصل النانو المصنع بايولوجيا بجهاز الطرد المركزي والغسل عدة مرات بالماء المقطر منزوع الايونات قبل أن يتم تجفيفه في الفرن بدرجة 60 مئويه ولمدة 30 ساعه. بعد ذلك تم حفظه لأجراء الفحوصات اللاحقه. التشخيص الفيزيائي والبايولوجي لجسيمات الفضه النانويه كان بعدة طرق منها الطرق الفيزوكيميائيه وتتظمن فحوصات: UV-vis. spectroscopy, XRD,TEM, SEM, AFM
أظهرت نتائج قياس الطيف المرئي للاشعه فوق البنفسجيه لعينات التجربه طيف قوي بين 444 و489 نانومتر دليل على تكون الجزيئات النانويه. وظهرت جسيمات الفضه النانويه المنتجه مختبريا بشكل جسيمات فضيه متناهية الصغر كروية الشكل كرستاليه مع وجه مركزي مكعب مع قمم تتوافق مع المستويات الشبكيه لجسيمات الفضه النانويه التي تم أنشائها خارج الخليه في فحص XRD .
أما في المجهر الألكتروني الماسح تظهرجسيمات الفضه النانويه بقطر من 70 – 20 نانومتر وتكون كرويه وموحده . ويظهر الفحص قمم للفضه عاليه وواضحه وحاده الجزيئات الملتصقه بسطح الجسيمات النانويه بأستخدام التحليل الطيفي للأشعه السينيه المشتته للطاقه تسببت في تحويل أيونات الفضه الى عنصر الفضه. أما في مجهر القوه الذريه يظهر أن أعلى معدل لقطر الجسيمات النانويه 32نانوميتر المصنع من بكتيريا

. *M.lacticum*

أما بايولوجيا فتم أختبارفعالية الجسيمات النانويه المصنعه في المختبر على البكتيريا المرضيه الموجبه والسالبه لصبغة غرام المعزوله من مستشفى الصدر التعليمي في ميسان وكان لها تأثير قوي على هذه الممرضات وبدرجات متفاوته حسب نوع الفضه النانويه المنتجه من قبل أنواع مختلفه من البكتيريا. أعلى معدل تثبيط للجسيمات النانويه كان حوالي (49) ملم لل *S.hominis*.

وايظا تم أستخدام هذه الجسيمات النانويه كمضاد للأورام وفي هذه الدراسه تم تجربتها على خط خلايا سرطان الثدي في المختبروتم تسجيل أقصى نشاط مضاد للسرطان عند تركيز 100ميكروغرام/مل حيث تم تثبيط (81.73%) من خلايا سرطان الثدي للنانوالمخلق من الأكتينومايسيتزمن نوع (M.*lacticum)*

بعد 72 ساعه من الحضانه . أيضا تم تجربة جسيمات الفضه النانويه المصنعه من بكتيريا الستريبتومايس *S.venezuelae* على نفس خط خلايا سرطان الثدي وتم تسجيل نسبة تثبيط مرتفعه (81.22%).

وتم تسجيل أقل نسبة تثبيط لنمو الخلايا السرطانيه عند تركيز 10ميكروغرام/مل ولنفس النانو المصنع من نفس البكتيريا يشير ذلك الى ان مع أرتفاع تركيز الجسيمات النانويه تزداد سميته على الخلايا السرطانيه. وكان نصف الخلايا المثبط لكلا عينتي الجسيمات النانويه المذكوره أعلاه 24.93 مايكروغرام لكل مل ،و 21.96مايكروغرام لكل مل ،على التوالي .

وبذلك أظهرت جسيمات الفضه النانويه المصنعه حيويا في المختبر نشاط فعال ومرتفع كمضاد لخط خلايا سرطان الثدي وكذلك كمضاد للبكتيريا المرضيه في رسالتنا الموسومه أعلاه .

وزارة التعليم العالي والبحث العلمي

جامعة ميسان

كلية العلوم

التخليق الحيوي لجسيمات الفضه النانويه بواسطة البكتيريا الشعاعيه وبكتيريا التربه وتحديد فعاليتها كمضاد للبكتيريا ومضاد للسرطان

رساله مقدمه الى

مجلس كلية العلوم/جامعة ميسان

كجزء من متطلبات نيل شهادة الماجستير

في علوم الحياة

من قبل

ثريا محبس ديوان

بكلوريوس علوم حياة/جامعة البصره (2001)

بأشراف

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2022 A.D 1443 A.H