BIOSYNTHESIS OF BIOACTIVE SILVER NANOPARTICLES BY *Actinomycetes* sp. AND ITS APPLICATION AS ANTIBIOTICS AGAINST SOME SELECTED BACTERIAL PATHOGENS

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IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE (B.Sc.) IN MICROBIOLOGY

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DECLARATION

I hereby declare that the project report was written under the supervision of DR. ADEBAMI G. E. and it is a product of my own research work. Information derived from various sources has been duly acknowledged in the text and the list of references provided. This researched project report has not been presented for the award of any degree.

Ademola-Phillips Omolola C. _____

Signature and Date

CERTIFICATION

This is to certify that this research project titled "**BIOSYNTHESIS OF BIOACTIVE SILVER NANOPARTICLES BY *Actinomycetes* sp. AND ITS APPLICATION AS ANTIBIOTICS AGAINST SOME SELECTED BACTERIAL PATHOGENS**'' was carried out by ADEMOLA-PHILLIPS, Omolola Christiana, with Matriculation number 18010101024. This report meets the requirements governing the award of Bachelor of Science (B.Sc.) Degree in Microbiology, Department of Biological Sciences of the Mountain Top University, Ogun State, Nigeria and is approved for its contribution to knowledge and literary presentation.

Dr. Adebami G.E. _____

(Supervisor) Signature / Date

Dr. (Mrs) C. I. Ayolabi _____

(Head of Department) Signature/Date

DEDICATION

This report is dedicated to the Almighty God and to my parents, Mr. and Mrs. Ademola-Phillips.

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ABBREVIATIONS

AgNPs	Silver nanoparticles
NPs	Nanoparticles
FTIR	Fourier Transform Infrared Spectroscopy
UV-vis	Ultraviolet- visible Spectroscopy
TEM	Transmission Electron Microscopy
SCA	Starch Casein Agar
NTA	Nanoparticle Tracking Analysis
XRD	X-ray Diffusion
ESBL	Extended Spectrum Beta-Lactamase
SPR	Surface Plasmon Resonance
SEM	Scanning Electron Microscope
DLS	Dynamic Light Scattering

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ABSTRACT

Nanotechnology is the novel science that deals with the production, manipulation and application of nano-materials. Nanoparticles can be produced by physical, chemical, or biological methods. Of these methods, biological is the most preferred due to its eco-friendly nature, and the fact that it is cheaper to carry out. This study is aimed at isolating, screening, and synthesizing silver nanoparticles using *Actinomycetes* sp. from MTU dumpsite. The isolates were screened for antibiotics production. The best isolate was identified using morphological and biochemical characterizations. Biosynthesis of silver nanoparticles using the selected isolate was carried out. Characterizations of the biosynthesized silver nanoparticles (AgNPs) using visual observation, UV-spectrophotometer, and FT-IR spectroscopy were investigated. The produced AgNPs was used as an antibacterial agent against five selected pathogenic bacteria including pathogenic *Escherichia coli*, pathogenic *Staphylococcus aureus*, *Proteus mirabilis*, *Salmonella enterica*, and *Enterococcus faecalis*. Out of the 13 bacterial isolates, only seven possessed antibacterial activities, out of which, isolate MDC4 and MDN9 identified as *Actinomycetes* sp were able to synthesize AgNPs. Colour change from yellow to dark brown, and 400 nm surface plasmon resonance (SPR) peaks between 24 to 72 hours were obtained. FT-IR showed 15 peaks with ten functional groups contributing to the synthesis of AgNPs. The biosynthesized AgNPs by the two isolates showed improved inhibitory effect against the selected pathogenic bacteria compared to the bacterial extract. Isolate MDC4 showed better inhibitory activity against pathogenic bacteria compared to MDN9. The biosynthesized AgNPs by MDC4 showed improved inhibitory effect compared to the bacterial extract, and chloramphenicol antibiotics against *S. enterica* 17, 15 and 0 mm), *S. aureus* (14, 13 and 11 mm), *E. coli* (19, 16 and 11 mm), *Proteus* sp. (27, 0 and 0 mm) and *E. faecalis* (13, 0 ad 0 mm) respectively, while that of MDN9 were *S. enterica* (15, 0 and 0 mm), *S. aureus* (12,0 and 0 mm), *E. coli* (45, 12 and 30 mm), *Proteus* sp. (no zones) and *E. faecalis* (no zones). Isolate. Therefore, MDC4 isolate can be used for antibiotics production on a larger scale.

Keywords: Actinomycetes, Antimicrobial, Nanoparticles, Nanotechnology, Pathogenic, Silver

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Actinobacteria is a group of microorganisms which are Gram-positive and are known to have a high guanine and cytosine content. They are unicellular and lack a defined cell wall and they produce and aseptate mycelia (Ranjani *et al.*, 2016). They share the characteristics of bacteria and fungi. Based on scientific classification, actinomycetes is a member of the order Actinomycetales, under the class Actinomycetia and the phylum Actinomycetota. The three main genera under Actinomycetes are: Actinomyces, Nocardia and Streptomyces (Gillespie, 1994).

Actinomycetes are Gram-postive, aerobic, spore-forming bacteria (Bhatti *et al.*,2017*)*. They possess the ability to produce antibiotics and other important therapeutic compounds (De Simeis and Serra, 2021). The spore-forming characteristic of actinomycetes supports the production of nanoparticles.

Nanotechnology is the novel science that deals with the production, manipulation and application of nano-materials, which are materials of dimensions in the nanoscale (Nasrollazadeh *et al.*, 2021). Nanotechnology is multidisciplinary in nature as it involves other disciplines such as: biology, chemistry and quantum mechanics (Alagarasi, 2011). Nanoparticles are particles whose size range between 0.1 - 100 nanometres. They are tiny particles and can be classified based on their shapes and sizes which serves as a basis of their properties. They have a number of optical, physicochemical and electrical properties which allows them to be applied in sensing, imaging, antimicrobial activities. And of all three methods, the biological has posed to be the most preferred as it is eco-friendly, it does not make use of toxic chemicals and it is economical. The biological method involves the use of bacteria, fungi, algae and plant extracts.

1.2 Statement of Problem

Antibiotics resistance is a rapidly emerging worldwide issue. This occurs when microorganisms are no longer susceptible to antibiotics. This poses an urgent problem to public health globally. A number of factors contribute to the development of resistance in microorganisms such as mutation, excessive drug use and incomplete drug dosage amongst others. Over the years, due to the widespread use of antibiotics, resistant strains of microorganisms have developed, and this has caused severe difficulties in treating illnesses caused by these microbes (Cesur and Demiroz, 2013). This phenomenon has consequences

such as treatment failures leading to inevitable loss of lives and the need for more expensive alternative treatment options to curb the increasing mortality rate (Nwobodo *et al.*, 2022).

1.3 Justification

The benefits of microbially-produced antibiotics cannot be overemphasized. Antibiotics have proven reasonably efficient in treating pathogenic infections but over time resistant microorganisms have emerged. A cheaper, readily available and more effective treatment option for pathogens is biosynthesized silver nanoparticles. They possess unique physical and chemical properties which aids their application in the biomedical field. Silver nanoparticles have proven to be biocidal against various drug resistant path This serves as a different therapeutic option to regular antibiotics, which has proven effective.

1.4 Aim and Objectives of the Study

The aim of this research is to synthesize silver nanoparticle using *Actinomycetes* sp. and apply same for the inhibition of pathogenic bacteria

i. To isolate Actinomycetes sp. from soil samples
ii. To screen the isolates for the synthesis of silver nanoparticles
iii. To identify the selected isolates using morphological, biochemical and characterizations.
iv. To determine the antimicrobial activity of the synthesized silver nanoparticles against selected pathogenic microorganisms.

CHAPTER TWO
LITERATURE REVIEW

2.1 Nanoparticles

Nanoparticles is a novel science that deals with the production, manipulation and application of nano-materials, which are materials in the nanoscale. Nanotechnology basically deals with design, production and characterization on nano sized materials (Krishna, 2017), including nanoparticles, nanotubes, nanowires, etc. Nanoparticles are particles ranging from the sizes 1-100nm. These particles are basically small objects that act as a whole unit in accordance with their transport and properties (Krishna, 2017). They exhibit some properties that qualify them as "ideal" such as nearly identical intensity, effective surface area, and discrete energy levels that can lead to some important adjustments to electronic properties (Daniel and Astruc 2004; Kato, 2011). Nanoparticles can be produced by physical, chemical and biological methods- use of biological systems such as bacteria, actinobacteria, fungi, viruses, algae, yeasts, plants (Pandit *et al.,* 2022). Biologically, nanoparticles are produced by intracellular and extracellular methods.

There are various types of nanoparticles, namely: Metallic nanoparticles, Metal-oxide nanoparticles, Sulfide nanoparticles and organic nanoparticles.

2.1.1 Properties of Nanoparticles

Nanoparticles have different properties; physical, electrical, thermal, magnetic and optical. Nanoparticles possess a number of physical and chemical properties.

2.1.1.1 Physical Properties

The physical properties of nanoparticles include the optical, magnetic, electrical and thermal properties. A physical property of nanoparticles is the size. Nanoparticles have their sizes in nanometers ranging from 1-100 nm. They are tiny and can only be viewed and characterized by employing the following techniques: Use of Scanning Electron Microscope (SEM), Transmission Electron Microscopy (TEM), X-ray Diffraction Microscopy, Dynamic Light Scattering (DLS) and Fourier Transform Infrared Spectroscopy (FTIR) (Fariq *et al*., 2017). Due to the small size of nanomaterials, the surface area to volume ratio is very large, and leads to large surface or interface atoms, leading to more "surface" dependent material properties. (Alagarasi, 2011). Basically, a greater amount of the atoms is found at the surface than the inside, giving them a larger surface area. The optical properties such as light absorption, reflection, penetration phosphorescence and luminescence abilities (Fariq *et al*., 2017) are

dependent on their size, shape and surface characteristics. These optical properties are applied in optical detectors, laser, sensor, imaging, display etc. (Alagarasi., 2011). The optical and electronic properties of NPs are interdependent to greater extent (Fariq *et al*., 2017). The optical properties of nanoparticles also include their colours. Magnetic properties of nanoparticles are more effective at a low dimension, between 10-20nm. The distorted electronic distribution in nanoparticles leads to magnetic property (Fariq *et al*., 2017). Mechanical properties of nanoparticles include elasticity, ductility tensile abilities and flexibility (Ealias *et al*., 2017). It also includes stress, strain, adhesion and friction Nanoparticles are capable of conducting heat more than liquids or solids. Therefore, the fluids containing suspended nanoparticles are predicted to show considerably more advantageous thermal conductivities relative to the ones of regular heat transfer liquids (Fariq *et al*., 2017). Heat transfer occurs at the surface hence it is better to use nanoparticles with large total surface area for heat transfer. Electrical properties of nanoparticles include: conductivity, semi-conductivity and resistivity. These properties have paved way for the application of nanoparticles in new-age electronics, thermal conductivity in renewable energy applications (Ealias *et al*., 2017).

2.1.1.2 Chemical Properties

The chemical properties such as the way nanoparticles react with the target and how sensitive it is to factors such as moisture, atmosphere, heat and light determine how it is applied (Ealias at al., 2017). Other chemical properties include: corrosive, anti-corrosive, oxidation, reduction and flammability properties (Ealias *et al.,* 2017).

2.2 Types of Nanoparticles

Nanoparticles are classified based on their physical and chemical characteristics. Based on their physical and chemical characteristics, they are classified into 5 classes of Nanoparticles, which are: Metallic Nanoparticles, Carbon-based Nanoparticles, Ceramic Nanoparticles, Semi-Conductor Nanoparticles, Polymeric Nanoparticles and Lipid-based Nanoparticles.

2.2.1 Metallic Nanoparticles

These nanoparticles are mainly composed of metal ions. Metal nanoparticles (MNPs) have a core made of an inorganic metal or metal oxide and are usually covered with a shell made of an organic or inorganic material or metal oxide (Khan *et al.,* 2020). Due to the known properties of localized surface plasmon resonance (LSPR), these NPs have unique photoelectric properties (Fariq *et al*., 2017). The common metallic nanoparticles are: Gold,

Silver, Zinc and Copper as they are easily biosynthesized. These nanoparticles are applied in many areas such as environmental, medicine and biomedicine, food and agriculture.

2.2.2 Carbon Based Nanoparticles

This are nanoparticles made from carbon precursors and their derivatives which include: fullerenes and carbon nanotubes. Fullerene is a carbon allotrope made of carbon atoms bonded together to form a hollow, spherical mesh. They have been applied in biomedicine in x-ray imaging and drug delivery. Carbon nanotubes are also carbon allotropes. They've sparked significant commercial interest in nanocomposites for a variety of uses, including fillers (Saeed and Khan, 2014; 2015).

2.2.3 Ceramic Nanoparticles

Ceramic nanoparticles are inorganic solids made of oxides, carbides, carbonates and phosphates that are manufactured by heating to high temperatures and then quick cooling (Fariq *et al*., 2017). They are chemically non-reactive and can withstand high temperatures. They are applied in various fields such as biomedicine in which they are used in imaging, also in drug delivery.

2.2.4 Polymeric Nanoparticles

Polymeric nanoparticles are made from an organic precursor. Polymer nanoparticles (NPs) are particles that can be packed with active compounds embedded in the interior of a polymer core or adsorbed on the surface (Zielińska *et al.,* 2020). Polymeric NPs have showed considerable prospective in the delivery of medications to specific locations in the human body for the treatment of a variety of ailments (Zielińska *et al.,* 2020). They are of two types depending on the technique of preparation: Nanospheres and Nanocapsule. They are used as drug delivery agents to deliver drugs, light, heat or other substances to cancer cells.

2.2.5 Lipid-Based Nanoparticles

Lipid- based nanoparticles includes: Liposomes, Solid Lipid Nanoparticles and Nanostructured Lipid Carriers (NLC) that are applied in cancer therapy and development of drugs. These nanoparticles may carry both hydrophobic and hydrophilic molecules, have extremely little or no toxicity, and extend the duration of pharmacological activity by having a longer half-life and regulated drug release (Garcia-Pinel, 2019).

2.3 Synthesis of Nanoparticles

Nanoparticles can be produced or synthesized by different methods. It could be physical, chemical or biological. Of all three techniques, the biological method is most preferred for

synthesizing nanoparticles because it is much safer, it does not produce toxic wastes that cannot be disposed, it is much cheaper and easier to set up and it is less laborious than the other methods.

2.3.1 Physical Methods

The physical methods of nanoparticles production include the use of force to crush, reduce larger bulk materials into nanoparticles. The physical methods are mostly top-down methods, i.e., bulk material-to- nanoparticles. They are: mechanical milling, nanolithography, sputtering, laser ablation, physical vapor deposition (PVD). These various methods cannot be employed in industrial or large-scale production because they require higher energy inputs and yields low amounts of nanoparticles. Generally, they are nor very efficient. However, these processes typically require sophisticated equipment, chemicals, radiant heat, and highpower consumption, which increases operating costs (Khandel *et al.,* 2018).

2.3.1.1 Mechanical Milling

Mechanical milling, grinding or attrition is a top-down synthesis method of nanoparticles whereby various elements are milled in a non-reactive atmosphere. It produces amorphous or crystalline nanoparticles. This process is carried out using planetary balls, tumbler mills or high energy shaker. The shear action of the balls during grinding produces the nanoparticles (Alagarasi, 2011). oxidation of the powdered nanoparticles, it should be handled in a vacuum system or glove box. The size, number and speed of the balls all affect the energy that would be transferred to the nanoparticles (Alagarasi, 2011).

2.3.1.2 Laser Ablation

Laser ablation is a frequently used method of synthesizing nanoparticles form solvents (Ealias *et al.,* 2017). A metal is irradiated with a laser beam while submerged in a liquid solution, it condenses a plasma plum that forms nanoparticles (Amendola *et al.,* 2009). It serves as an alternative to chemical reduction of metals to form nanoparticles, it provides a stable production of nanoparticles in organic solvents and does not require any form of chemicals (Ealias *et al.,* 2017).

2.3.1.3 Sputtering

This is the deposition of nanoparticles on a surface by ejecting particles from the surface through collision with ions (Shah *et al.,* 2006). It involves deposition of a thin layer of nanoparticles followed by annealing. Some factors that affect the size and shape of the

nanoparticles include: the thickness of the layer, annealing temperature, annealing duration and the type of the substrate (Lugscheider *et al.,* 1998).

2.3.1.4 Nanolithography

Nanolithography is a method of producing nanoscale materials using various processes; optical, electron-beam, multiphoton, nanoimprint and scanning probe lithography (Ealias *et al.,* 2017). The process involves printing a required shape on a light sensitive material that carefully removes a portion of the material to create the desired shape and structure (Ealias *et al.,* 2017). It is expensive to run and maintain.

2.3.1.5 Physical Vapor Deposition

Physical vapor deposition is a combination of procedures used to fabricate nanoparticles and deposit thin layers within the size scale of a few nanometers to many micrometers. This method is eco-friendly and it consists of three major steps: vaporization of the material from a strong source, transportation of the disintegrated material and nucleation and development to create films of nanoparticles (Ma *et al.,* 2014). Other techniques used in physical vapor deposition include: sputtering, electron beam evaporation and pulsed laser deposition (Prabrakaran *et al.,* 2021).

2.3.2 Chemical Methods

The chemical methods of nanoparticles production involve reducing metal ions in chemical solutions. The methods are mostly bottom-up synthesis methods, i.e., building up of nanoparticles starting from atoms to clusters then nanoparticles (Ealias *et al.,* 2018). They include: sol-gel process, and micro-emulsion method, chemical vapor deposition (CVD), electrochemical reduction amongst others. The chemicals are applied in the reduction process, other chemicals which serve as solvents are also used. These chemicals are non-biodegradable and are toxic. In addition, some toxic chemicals can contaminate the surface of nanoparticles, making them unfit for certain biomedical applications (Khandel *et al.,* 2018).

2.3.2.1 Sol-Gel Process

Sol is a solution of solids suspended in a liquid phase, forming a colloid. The gel is a solid molecule submerged in a solvent. This process is simple and can synthesize most of the nanoparticles. This is a wet chemical process that contains chemical solutions that act as precursors to an integrated system of individual particles. Metal oxides and metal chlorides are precursors commonly used in the sol-gel process (Ramesh, 2013). The precursor is then

dispersed in the host liquid by either shaking, stirring, or sonication, and the resulting system contains a liquid phase and a solid phase. Phase separation is performed using various methods such as sedimentation, filtration, centrifugation, etc. to recover the nanoparticles, and further water removal by drying (Mann *et al.,* 1997).

2.3.2.2 Micro-Emulsion Method

Micro-emulsion method is employed in preparing the polar phase (water), non-polar phase (hydrocarbon) and surfactant- serves as a layer between the water and hydrocarbon. There are 2 common types if micro-emulsion techniques used: water-in-oil and oil-in-water, depending on the type of surface-active agent used (Palomo *et al.,* 2019). Gold nanoparticles were produced using the water-in-oil micro-emulsion system with a standard diameter of 11-12nm (Maria *et al.,* 2012).

2.3.2.3 Chemical-Vapor Deposition

This method involves depositing a thin layer of gaseous reactants onto a substrate, in a reaction chamber at an optimum temperature through the combination of gas molecules. A chemical reaction takes place when a heated substrate makes contact with the combined gas molecules (Bhaviripudi *et al.,* 2007). The chemical reaction produces a thin layer of nanoparticles on the surface of the substrate which is recovered and used (Ealias *et al*, 2017).

2.3.2.4 Electrochemical Method

All reactions rely on electricity and are commonly used on large scale. An electric field is created between two electrodes in this approach, and reduction happens or the metal precursor dissolves into the solution at the metallic anode, resulting in nanoparticles at the negative pole. Also included is a reagent for stabilizing the new nanoparticles. The benefits include being able to control the size, being environmentally friendly, and being cost effective (Huynh *et al.,* 2020).

2.3.3 Biological Method

The biological method of synthesizing nanoparticles involves the use of biological systems such as microorganisms (bacteria, fungi, virus, yeast, algae) and plants. This method is preferred to the other methods of producing nanoparticles because it is much safer, less laborious, environmentally friendly, and cheap to setup and it yields higher amounts of nanoparticles (Ravindra *et al*. 2012; Prasad 2014). Green synthesis of nanoparticles has a good number of advantages over physical and chemical methods. This method is low cost, environmentally friendly, consumes less energy, and can produce nanoparticles in clearer size

and morphology with widespread compatibility with medical, agricultural and environmental applications (Gour *et al.,* 2019). Bacteria and fungi are most commonly used to biosynthesize nanoparticles. Bacteria are suitable for the process because: they can be grown on inanimate media with an optimum growth rate, they can adapt in high metal conditions and they have the ability to reduce metal ions into nanoparticles. Fungi can be employed to biosynthesize nanoparticles because they grow faster than bacteria under the same conditions, the fungi mycelia offer a wider surface area for interaction. Also, they produce a larger number of enzymes so the bioreduction of metals into nanoparticles is faster. Examples of fungi species employed in nanoparticles reduction are: *Aspergillus niger, Candida utilis, Candida albicans, Penicillium chrysogenum, Trichodrema versicolour* etc. Generally, nanoparticles are biosynthesized when the microorganisms take target ions from their environment and reduce the metal ions into the elemental metal through enzymes produced by the cell. (Li *et al.,* 2011). The biological synthesis of nanoparticles can be further divided into two methods: Intracellular and Extracellular method.

2.3.3.1 Intracellular Method

The intracellular method involves synthesizing the nanoparticles within the cell, it makes use of the cell's mechanism. The metal ions are absorbed into the microbial cell to form microorganisms using enzymes. The nanoparticles are produced within the cell and give resulting chromatic changes. The microorganism is grown in appropriate liquid culture, the microbial biomass is washed with distilled water. The resulting mixture is centrifuged to obtain the microbial biomass at the bottom of the vessel (pellet). The microbial biomass is reacted with an aqueous metal solution. The solution of the microbial biomass and aqueous solution is incubated at appropriate conditions till a specific chromatic change is observed. (Koul *et al.,* 2021). The metal ions are absorbed into the microbial cell through cationic membrane transport systems that transport metabolically important cations. (Messaoudi and Bendahou, 2020). The metal ions are bio-reduced by the enzymes naturally occurring within the cytoplasm of the cell to form clusters of nanoparticles. The appearance of a whitish yellow to yellow colour indicates the presence of zinc and manganese nanoparticles, appearance of pale yellow to pinkish colour indicates the presence of gold nanoparticles and the appearance of pale yellow to brownish colour indicates silver nanoparticles (Koul *et al.,* 2021).

2.3.3.2 Extracellular Method

The extracellular method involves the synthesizing of nanoparticles outside the cell wall and not within. It does not require the cellular mechanism; it is mediated by enzymes on the cell

membrane or enzymes released into the growth medium. The microorganisms are grown and maintained in suitable broth. The broth containing the microbial cell is centrifuged to separate the supernatant which contains reductase enzymes. The cell-free supernatant containing the reductase enzyme is reacted with the aqueous solution of metal ions in a different vessel. The metal ions are trapped outside the cell and are not absorbed into the cell through the electrostatic interaction of the positively charged ions with the negatively charged cell wall. The enzymatic reduction of the metal ions results in the formation of nanoparticles (Koul *et al.,* 2021).

2.4 About Silver Nanoparticles

Silver nanoparticles can be categorized under metallic nanoparticles. They have been found to be very applicable in areas such as: nano crystalline silver dressings, creams, gels that efficiently reduce bacterial infections in chronic wounds (Ip *et al.,* 2006). They have been discovered to possess effective antibacterial activity. Silver as a metal provides a most efficient antimicrobial activity against microorganisms (Mohamed and Ahmed, 2016). Silver nanoparticles are capable of killing bacteria intracellularly and extracellularly, they also show activity against multi-drug resistant Gram-positive and negative bacteria (Roe *et al.,* 2008; Zeng at al., 2007).

2.5 Characterization of Nanoparticles

There are different techniques for determining the various characteristics of nanoparticles. They include: Scanning Electron Microscope (SEM), Transmission Electron Microscope (TEM), X-ray Diffraction Microscopy (XRD), Dynamic Light Scattering (DLS) and Fourier Transform Infrared Spectroscopy (FTIR).

2.5.1 Scanning Electron Microscope (SEM)

Scanning electron microscope is a very-high resolution technique used to characterize and view nanoparticles using a ray of high-energy electrons. It makes use of electron scanning principles to give the properties of nanoparticles. It is used to give the morphological and topological properties of nanoparticles. This technique uses a beam of electrons with accelerated voltage to scan the surface of the nanoparticles. It gives an image of the surface of the nanoparticles, more like a two-dimensional imaging technique. It is able to give the size, shape and surface topology of the nanoparticles at the micro or nanoscale. Its limitation is that it is not capable of capturing the internal structure of the nanoparticles (Khandel *et al.,* 2018). Mirazdeh and Akhbari, (2016) used SEM to study the properties of zincoxide modified frameworks which

showed that the zincoxide nanoparticles and morphologies of the modified frameworks at different reaction conditions.

2.5.2 Transmission Electron Microscope (TEM)

Transmission electron microscopy is used to characterize metallic nanoparticles (Khandel *et al.,* 2018). It uses a short wavelength beam of electrons projected to a phosphor screen to obtain an image of the nanoparticle. This technique provides information on nanoparticle topography, monodispersity, composition, and crystallinity (Khandel *et al.,* 2018). It makes use of electron transmittance principle to give the necessary information about. It also gives needed information about multilayer samples such as the quadropolar hollow shell structure of CO_3O_4 nanoparticles (Fariq *et al*., 2017). Kim *et al.* (2016) used transmission electron microscopy to characterize the morphology of methyl-functionalized cobalt-ferrite-silica nanoparticles and to estimate the size of the magnetic core. The nanoparticles had a size of about 300nm. The structure of the nanoparticle was observed to have two layers; the inner was the magnetic core of cobalt ferrite dispersed in mesoporous silica and the outer layer was composed of bare mesoporous silica.

2.5.3 X-Ray Diffraction Microscopy

This is a non-destructive technique used to identify the crystalline phase of nanoparticles (Khandel *et al.,* 2018). It makes use of X-rays of fixed wavelength which is illuminated onto the sample, the strength of the reflected radiation is recorded using a goniometer (Aziz *et al.,* 2015). Upadhyay *et al.* (2016) used X-ray line broadening to determine the average crystalline size of magnetic nanoparticles which was observed to be in the range of 9-53nm.

2.5.4 Dynamic Light Scattering (DLS)

Dynamic light scattering gives the sizes of the nanoparticles at extremely low level. This technique was used to determine the size of silica nanoparticles with absorption proteins from serum and it was observed that the increase in size was directly proportional with the increase in number of protein layers (Sikora *et al.,* 2016). Erjaee *et al.* (2017) employed DLS in the biosynthesis of silver nanoparticles to measure the peak of absorbance at each trial while varying a number of reaction parameter. The peak of absorbance was measured for each trial time while the parameters were varied for each trial.

2.5.5 Fourier Transform Infrared Spectroscopy (FTIR)

This technique is used to identify the types of functional groups or chemical bonds present in a sample, based on their specific unique absorption signatures by measuring the ability of the

chemical bond to stretch and bend through energy absorption (Khandel *et al.*, 2018). FTIR provides information on the surface chemistry of nanoparticles by identifying functional groups attached to the surface of the metal nanoparticles because they exhibit the same absorption pattern as the corresponding free radicals (Khandel *et al.*, 2018). Erjaee *et al.* (2017) used FTIR to identify the functional groups of biosynthesized silver nanoparticles.

2.6 Application of Nanoparticles

Nanoparticles can be applied to improve four major areas of life. They can be employed to improve processes in these four major areas and improve the yield of specific products. These four major areas include: Biomedicine, Environment, Agriculture and Industrial.

2.6.1 Applications in Biomedicine

Nanoparticles can be applied in various areas of biomedicine. They can be used to improve the efficiency of antibiotics, in drug delivery and cancer treatment and management, diagnosis of diseases. Nanoparticles possess antibacterial properties; they can serve as antibiotics. The biologically synthesized nanoparticles have a larger surface area and smaller size. These properties allow NPs to effectively interact with microbial cell membranes and enter cells to interfere with DNA replication and metabolic pathways (Fariq *et al.,* 2017). The synergistic effect of silver nanoparticles synthesized from *Rhizopus stolonifer* with antibiotics like ciprofloxacin, nitrofurantoin and carbenicillin against bacterial species of Enterobacteriaceae family was studied and the reducing order of antibacterial activity was obtained. Nitrofurantoin had the highest efficacy (50%) followed by carbenicillin (33.56% efficacy), then ciprofloxacin (30.53% efficacy) (Banu *et al*, 2011). In some cases, nanoparticles display greater antibacterial properties than conventional antibiotics. For example, the antibacterial property of silver nanoparticles biofabricated from *Bacillus cereus* against some pathogenic bacteria species: *Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa, Escherichia coli and Salmonella typhii,* using the agar-well diffusion method. The nanoparticles were observed to produce greater zones of inhibition than the conventional antibiotics – amoxicillin, streptomycin and ofloxacin. (Sunkar *et al,* 2012).

Nanoparticles can be employed in the treatment of cancer through targeted drug delivery to the affected organ. Nanoparticles can be successfully employed for tumor diagnosis and treatment through targeted delivery (Koul *et al.,* 2021). The efficacy of silver nanoparticles biosynthesized from *Cryptococcus laurentii* was tested and observed to possess effective anticancerous and antitumor abilities against breast cancer cells (Ortega *et al.,* 2015).

Biofabricated selenium nanorods from *Streptomyces bikiniensis* strain EssamA-1 was tested against human cancer cell lines and observed that the nanorods destroyed the HEP-G2 and MCF-7 cancer cells (Ahmad *et al.,* 2015). Nanoparticles can also be used as carrier vehicles in targeted drug delivery to transport the drugs to the target site in the body. Nanomaterials commonly used in drug delivery includes: liposomes, nanospheres, polymeric micelles, water-soluble polymers, nano-emulsions -and NP-coated natural antibodies. (Salouti *et al.,* 2014). The water-soluble materials enhance diffusion of the drugs into the target cells. (Koul *et al.,* 2021). Targeted drug delivery using NPs reduce the risk of side-effects of cancer drugs caused by the drug's toxicity (Koul *et al.,* 2021). NPs can offer cost effective, fast, specific and accurate detection of pathogens as well as chronic diseases (Koul *et al.,* 2021). Nanoparticles possessing fluorescent, metallic and magnetic properties can be applied in the imaging, characterization, identification and tracking of pathogens and diseases development (Tallury *et al.,* 2010). Fluorescent NPs can be used for viewing the early stage of chronic diseases such as cancer while Magnetic NPs can be applied in advance detection techniques such as MRI (Koul *et al.,* 2021). In identification of cancer cells, the NPs bind to the cancer cells specifically and help in differentiating the cancer cells from normal cells.

2.6.2 Applications in Environment

Nanoparticles can be employed in bioremediation to clean up pollutants like cationic dyes, acid dyes, azo dyes from the environment (Koul *et al.,* 2021). These pollutants cause water pollution and negatively affect aquatic life (Koul *et al.,* 2021). The general property of nanoparticles; small size but large surface area enhances their actions as catalysts or they adsorb the pollutants using the large surface area (Koul *et al.,* 2021). It was observed that silver nanoparticles are capable of efficiently decolourizing organic dyes (Sharma *et al.,* 2015). Both silver and gold nanoparticles are capable of decolourizing organic dyes in a short time and acting as a catalyst in the reaction (Suvith et. al 2014). Different forms and types of nanoparticles are employed in soil bioremediation, they are also more effective when they are biosynthesized. (Koul *et al.,* 2018). Nanoparticles can also be employed in air, water and surface disinfection (Shruti *et al.,* 2018). Silver nanoparticles can be employed to remove bacterial aerosols contamination. In enhancing this, carbon nanotubes can be used to increase the surface area of the nanoparticles (Jung *et al.,* 2011). Nanoparticles can be used to control water pollution to an extent, using porous silver nanoparticles to make water filters capable of trapping bacteria (Yakub *et al.,* 2012). They can be used to disinfect, purify and desalinate water. They can reduce heavy

metals, pathogens and other organic contamination in water. Nanoparticles have also proven to be effective in cleaning-up oil spills and treatment of municipal and industrial waste water (Ealias *et al.,* 2019).

Furthermore, nanoparticles can be applied in surface disinfection. When embedded in paints, they exhibit bactericidal properties on the painted surface. They can kill pathogenic bacteria such as: *Staphylococcus aureus and Escherichia coli* present on the coated surface (Kumar *et al.,* 2009). Silver nanoparticle coated paper can be employed in packaging food materials to increase the shelf-life by preventing bacterial growth (Agrawal *et al.,* 2018).

2.6.3 Application in Agriculture

In agriculture, nanoparticles can be employed in the manufacture of nanopesticides, nanofungicides and nanofertilizers. Metallic and metal oxide nanoparticles possess effective antifungal properties against fungal species affecting plants (Koul *et al.,* 2021). It has been observed that copper and copper oxide nanoparticles biosynthesized from *Streptomyces* spp. eliminated pathogenic plant fungi such as: *Alternaria alternata, Pythium ultimum, Fusarium oxysporum and Aspergillus niger* (Hassan *et al.,* 2019, 2018). The antifungal property of silver nanoparticles biofabricated from *Pseudomonas* spp. and *Achromobacter* spp. was tested against *Fusarium oxysporum* infection in chickpea. The nanoparticles displayed effective antifungal activity against the pathogen (Kaur *et al.,* 2018). As nanofertilizers, nanoparticles can serve as a safer alternative to chemical fertilizers, thereby preserving the soil fertility. Carbon-based nanomaterilas used as fertilizers proved that nanofertilizers could diminish the use of chemical fertilizers (Bisinoti *et al.,* 2019). Nanoparticles could be employed in the fabrication of pesticides. In nanopesticides, they exist in forms of micelles, particles, nanopolymers (organic constituent) and metal oxides (inorganic constituent) (Koul *et al.,* 2021).

2.6.4 Application in Industry

In the industry, nanoparticles can be applied in the synthesis of bioethanol – a metabolite. Bioethanol is a metabolite produced from the microbial fermentation of agricultural products such as sugarcane or corn. Nanoparticles can be employed in the fermentation to favor bioethanol production. They influence the biochemical conversion process by affecting either the enzymatic activity or the gas–liquid mass transfer rate (Kushwaha *et al.,* 2018). It was reported that nanoparticles improved bioethanol production by 166.1% with methyl-functionalized silica nanoparticles in syngas fermentation, and the only challenge was the

inefficient reuse of nanoparticles (Kim *et al.,* 2014). Syngas- short for synthetic gas is a gas mixture of hydrogen, carbon monoxide and some carbon dioxide. Syngas fermentation is a microbial fermentation process whereby microorganisms make use of a mixture of hydrogen, carbon monoxide and carbo-n dioxide as a carbon and energy source to produce fuel and chemical products such as: butanol, acetic acid, ethanol, butyric acid and methane. Nanoparticles can also be applied in bioethanol production is in the detection of compounds using metal nanoparticles that have been immobilized onto the nanosheet structure (Kushwaha *et al.,* 2018). Glassy carbon electrode modified with graphene oxide containing copper nanoparticles was used for the determination of total reducing sugars and it achieved better accuracy and reusability of the prepared system (Santos *et al.,* 2016).

2.7 Actinomycetes

Actinobacteria is a group of microorganisms which are Gram-positive and are known to have a high guanine and cytosine content. They are free-living and saprophytic (Raja nad John, 2016). They are unicellular and lack a defined cell wall and they produce and aseptate mycelia (Ranjani, 2016). They share the characteristics of bacteria and fungi (Chamikara, 2016). Based on scientific classification, actinomycetes is a member of the order Actinomycetales, under the class Actinomycetia and the phylum Actinomycetota. The three main genera under Actinomycetes are: Actinomyces, Nocardia and Streptomyces.

Actinomycetes are Gram-postive, aerobic, spore-forming bacteria. They exhibit branched filamentous growth and contain high guanine plus cytosine content in their nucleic acid -DNA (Chamikara, 2016). They are Gram-positive mycelial bacteria capable of producing various compounds important to the industry and medical field such as: fungicides, immunosuppressants and antibiotics (Kodzius and Gojobori, 2015). They possess the ability to produce antibiotics and other important therapeutic compounds. The spore-forming characteristic of actinomycetes supports the production of nanoparticles.

Actinomycetes are the most abundant microorganims in the soil, in which they form thread-like filaments or branching structures (Chamikara, 2016). They give the soil its characteristic earthly smell (Chamikara, 2016). When grown on agar, the branch forms a hyphae network that grows on the agar surface (aerial hyphae) and under the agar surface (substrate hyphae) (Dangi, 2014). The cell wall composition of actinomycetes varies among taxonomic groups (Dangi, 2014). The cell wall of actinomycetes is rigid enough to prevent bursting of the cell due to high osmotic pressure and maintain the cell shape (Chamikara, 2016). Most are non-motile, but when observed to be motile, they are flagellated spores (Dangi, 2014).

2.8 Classification of Actinomycetes

Actinomycetes are classified as bacteria, although they have fungi-like properties. Actinomycetes are included under the order Actinomycetales which is further divided into four families: Streptomycetaceae, Actinomycetaceae, Actinoplanaceae, and Mycobacteriaceae. The Bergey's Manual of Systematic Bacteriology has five volumes that contain the internationally recognized names and descriptions of bacteria species (Chamikara, 2016). Actinomycetes classification has been arranged as follows:

Volume 5 of the Bergey's Manual of Systematic Bacteriology divides the phylum actinobacteria into six classes namely: Actinobacteria, Acidimicrobiia, Coriobacteriia, Nitriliruptoria, Rubrobacteria and Thermoleophilia. The class actinobacteria is then further divided into 16 orders which are: Actinomycetales, Actinopolysporales, Bifidobacteriales, Catenulisporales, Corynebacteriales, Frankiales, Glycomycetales, Jiangellales, Kineosporiales, Micrococcales, Micromonosporales, Propionibacteriales, Pseudonocardiales, Streptomycetales, Streptosporangiales, Incertaesedis. Table 2.1 below shows the classification of Actinomycetes with their corresponding characteristics.

2.9 Application of Actinomycetes Genera

Actinomycetes can be used in various areas, both industrially and non-industrially. The most common industrial use of Actinomycetes is in antibiotics production. A great percentage of the known antibiotics are produced by Actinomycetes (Chavan *et al.,* 2013). Besides antibiotics production, Actinomycetes can be applied as: Biosurfactants, Enzyme Inhibitors, Immunomodifiers, Antifungals, Plant growth promoting agents, Biocontrol agents, Biopesticide agents, Producers of plant growth hormone. From the applications stated above, it can be clearly deduced that actinomycetes are very useful and can be applied in different areas.

Table 2.1: Characterization of Actinomycetes (Chandramohan, 1997)

Section	Characteristics
Nocardioform actinomycetes	Aerobic, may be acid-alcohol fast, cocci and branched filaments or form substrate and aerial mycelium that fragment; wall chemotype IV; contain mycolic acids.
Actinomycetes with multilocular sporangia	Aerobic to facultatively anaerobic; mycelium divides in all planes, no aerial hyphae, wall chemotype III.
Actinoplanetes	Aerobic sporoactinomycetes, nonmotile, spores may be enclosed within vesicles; no aerial mycelium; wall chemotype II; whole-organism hydrolysates contain arabinose and xylose.
Streptomycetes and related genera	Aerobic sporoactinomycetes; form an extensively branched substrate and aerial mycelium.
Thermonospora and related genera	Aerobic spordactinomycetes; form an extensively branched substrate and aerial mycelium, both of which may carry single chain of spores; spores which are either motile or non-motile; wall chemotype III.
Thermoactiomycetes	The stable filaments produce aerial growth. Single spores (endospores) are formed on both aerial and vegetative filaments. All species are thermophilic. The cell wall contains meso-DAP but no characteristics amino acids or sugars.
Other genera	They all produce aerial growth bearing chains of spores.

2.9.1 Actinomycetes as Antibiotics Producers

Actinomycetes are capable of producing antibiotics that differ in chemical structure and mechanism of action (De Semeis *et al.*, 2021). Different genera of Actinomycetes are also capable of producing the same classes of antibiotics (Grasso *et al.*, 2016). They produce various classes of antibiotics such as: β-lactams, Aminoglycosides, Tetracyclines, Macrolides, Peptides, Chloramphenicol, Nucleosides, Anthracyclines, Polyethers. Lincosamides, Aminocoumarins, Epoxides, Ansamycins (Chavan *et al.*, 2013 ; De Semeis *et al.*, 2021). The ability of Actinomycetes to produce secondary metabolites depends solely on the differentiation in cell morphology and physiology (De Semeis *et al.*, 2021). The genes responsible for production of antibiotics as well as other secondary metabolites are in the genome (De Semeis *et al.*, 2021). For a bacterium to be susceptible to an antibiotic, the membranes have to be permeable by the antimicrobial agent. Drugs such as: β-lactams and fluoroquinolones use pore-forming proteins called porins to pass through the cell membrane while larger antibiotics such as: macrolides, diffuse through the lipid bi-layers (De Semeis *et al.*, 2021).

2.9.2 Actinomycetes as Biosurfactants

Biosurfactants are surface-active chemicals produced by microorganism, they are molecules produced by microorganisms that have effect on surfaces (Chavan *et al.*, 2013). This biosurfactants have more advantages than the chemically synthesized surfactanats as they are less toxic, highly specific and are biodegradable. They are also easy to synthesize from cheap substrates, they are effective at extreme conditions (Chavan *et al.*, 2013). They have also been found to possess antimicrobial property against pathogenic microorganisms. In research by Afiriyanto *et al.* (2020), *Actinomycetes spp.* was isolated from mud areas, the organisms were screened for biosurfactants by testing their emulsification activity, surface tension activity and lipolytic activity. The antimicrobial activity of the biosurfactant was then tested against *E. coli* and *Staph. aureus*, in which it inhibited their growth. Actinomycetes are also capable of producing bioemulsifiers (Chavan *et al.*, 2013).

2.9.3 Actinomycetes as Enzyme Inhibitors

Enzyme inhibitors are substances that temporarily or permanently interact with enzymes in some way to slow down or stop an enzyme-catalyzed reaction from occurring. The three main categories of inhibitors are competitive, noncompetitive, and uncompetitive (Kuddus, 2019). Imada, (2005) isolated Actinomycetes strain from marine sediments that tested positive for the inhibition of the N-Acetyl-β-D-glucosaminidase enzyme which has an increased activity in

diabetic patients. The strain inhibited the enzyme by the production of two compounds; pyrostatins A and B, in the presence of seawater (Imada, 2005). In the same research, the isolates which were cultured on starch agar showed a purple zone of inhibition after the addition of amylase and iodine solution (Imada, 2005). This showed α- amylase inhibition by the isolates.

2.9.4 Actinomycetes as Enzyme Producers

Actinomycetes sp. are known enzyme producers (Elnahas *et al.,* 2021). They produce industrially important enzymes which are more stable and are substrate specific (Elnahas *et al.,* 2021). They produce extracellular enzymes capable of degrading organic matter (Mukhtar *et al.,* 2017). The enzymes include: cellulases, lipases, xylanases, amylases, proteases, chitinases, cutinases, α- Amylases (Mukhtar *et al.,* 2017; Elnahas *et al.,* 2021). These enzymes are applicable industrially in different processes. Cellulases are used in industrial waste treatment, colour brightening detergents, extracting colour from juices (Niehaus *et al.,* 1999; Bhat, 2000). Chitinase is industrially used to degrade chitin (Kunz *et al.,* 1992).

2.9.5 Actinomycetes in Bioremediation

Bioremediation is the use of soil microorganisms to eradicate or neutralize contaminants from an environment (Sharma, 2020). Various researches showed that *Streptomyces spp.* possess the ability to degrade hydrocarbons (Radwan *et al.,* 1998; Barabas *et al.,* 2001). A number of by generating cellulose- and hemicellulose-degrading enzymes, extracellular peroxidase, a variety of strains can solubilize lignin and lignin-related chemicals and destroy its constituent components (Mason *et al.,* 2002)

2.10 Pathogenic Microorganism

Pathogenic microorganisms refer to the microorganisms capable of establishing a disease in hosts. They can be transmitted directly or indirectly through different routes (Meena *et al*., 2019). These microorganisms include species of bacteria, protozoa, fungi and viruses. Food products provide a suitable environment for microorganisms to thrive as an evident source of high nutrients (Mendez *et al.,* 2020).

The infection of food products by microorganisms poses a worldwide issue as the growth of microorganisms in food can lead to foodborne diseases, food borne infection or food intoxication and food spoilage. The acceptability of a food product for consumption largely depends on the presence and type of microorganisms present in the food. Bacteria, molds and yeasts are the major causal organisms of food spoilage and intoxications (Blackburn, 2006).

2.10.1 Pathogenic Bacteria

Bacteria are one of the predominant pathogenic microorganisms. They survive in food because it is a nutrient rich source and the food temperature may be ambient for their growth. The ingestion of bacteria in food leads to food-borne illnesses. Bacteria cause spoilage in food by growing on or in the food, they do not often cause a colour change, odor change, taste or even texture change in the food product, making food contamination hard to recognize (Bahome *et al.,* 2012). The presence of bacteria in food products cause spoilage in forms of food contamination or food intoxication. When bacteria grow in food products, they produce toxins as a by-product of their growth and multiplication and a consumption of these toxins is known as food intoxication. These toxins do not necessarily alter the appearance of food products but when ingested they are capable of causing severe and irreversible damage in humans (Bahome *et al.,* 2012). Example of bacteria that produce toxins include: *Clostridium botulinum*, *Staphylococcus aureus* etc. Bacterial spores are also capable of growing at high temperatures and Gram-positive bacteria can grow in the presence of ambient air (aerobic) and in the absence of oxygen (anaerobic). Some anaerobic bacteria grow in canned or sealed processed foods and produce hydrogen sulfide which can also contribute to the spoilage of the food (Sevindik, 2021). Bacteria that grow at low temperatures are capable of producing gas and foul odors in foods such as: frozen meat products. Examples of common pathogens genera include: *Staphylococcus spp*., *Bacillus spp*., *Listeria spp*., *Clostridium spp*., *Escherichia spp*., *Campylobacter spp*., *Vibrio spp*., *Shigella spp*. etc (Allos, 1997; Adams and Moss, 2000; Bintsis, 2017; Jiang *et al.,* 2004).

2.10.1.1 *Bacillus cereus*

Bacillus cereus is a member of the *Bacillaceae* family, they are Gram-positive motile rods with the ability to form spores (Bacon and Sofos, 2003). Their common sources are soils, aquatic environments (Bintsis, 2017). The endospore forming cells grow optimally between temperatures of 30-40^{o}C. They grow best at pH of 4.9-9.3, this effect can be limited in food at lower pH (Bacon and Sofos, 2003). They have been found to grow at a minimum water activity of 0.912 (Rajikowski and Smith, 2001). They produce two types of toxins: the emetic toxin and the diarrhoeal toxin. The emetic toxin is produced during the growth phase in food while the diarrhoeal toxin is produced during the growth phase in the small intestine. (Bintsis, 2017). The emetic toxin is thermostable while the diarrhoeal toxin is thermolabile (Bintsis, 2017). The spores produced are adhesive using pilli or appendages, this makes them commonly present in food production areas making them easily transmittable to food (Bintsis, 2017). The diarrhoeal

and emetic toxins produce symptoms such as: diarrhoea and abdominal pain, nausea and vomiting respectively. Their ability to cause these symptoms implies that they survived the cooking or sterilization process (Bintsis, 2017).

In a study of *B. cereus* outbreaks (Bennett *et al.,* 2013), 50% of the illnesses were attributed to rice dishes, majorly fired rice which attributed for 68% of the illnesses. Rice which was frequently cooked and served immediately accounted for 42% or were part of large masses of foo accounted for 33% (Bennett *et al.,* 2013). Meat and poultry which were prepared and served immediately accounted for 50% of the outbreaks, the roasted meals accounted for 33% while those part of liquid or semisolid meals accounted for 17% of the outbreaks (Bennett *et al.,* 2013).

2.10.1.2 *Escherichia coli*

This is a Gram-negative, non-sporulating bacilli. It may or may not be motile with some rods being flagellated and some lacking the flagella (Mitscherlich and Marth, 1987). It is a facultative anaerobic organism, it is a fermenter of glucose to form by-products such as lactic, formic and acetic acids. They have an optimum pH of 6.0 to 8.0 (Mitscherlich and Marth, 1987). It is transmitted through the faecal-oral route and also through contamination of food products during slaughtering or processing of animals (Garcia *et al.,* 2010). Usage of manure from livestock as fertilizer for agricultural crops and vegetables can contaminate farm produce and irrigation water (Garcia *et al.,* 2010). E. coli can survive in the environment for long periods of time and can proliferate in vegetables and other foods (Bintsis, 2017).

Pathogenic *E. coli* can be categorized into six groups based on their mechanism for pathogenesis: Enteropathogenic *E. coli* (EPEC), Enterohemorrhagic *E. coli* (EHEC) also known as Shiga-toxin producing *E. coli* (STEC), Enterotoxigenic *E. coli* (ETEC), Enteroaggregative *E. coli* (EAggEC), Enteroinvasive *E. coli*, Attaching and Effacing *E. coli* (A/EEC) (Croxen *et al.,* 2013; Garcia *et al.,* 2010*).* Of all pathotypes, STEC has been found to be the most severe. In a study on *E. coli* pathotypes in Iran (Eybpoosh *et al.,* 2021), STEC was the most frequent pathotype as it was observed in all provinces of the country.

2.10.1.3 *Listeria monocytogenes*

Listeria monocytogenes belongs to the family Listeriaceae. It is found in moist environment, water, soil, decaying vegetation. It is one of the major causes of death from food-borne pathogens in pregnant women, the aged, immunocompromised persons and newborns (Buchanan *et al.,* 2017). It can be found in various raw foods and foods that become contaminated after cooking (Bintsis, 2017). It can spread from the intestines to the central

nervous system and the fetal-placental unit (Bintsis, 2017). It has thirteen serotypes: 1/2a. 1/2b, 1.2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e and 7. *L. monocytogenes* infection in the human system can cause septicaemia, gastroenteritis and meningitis (Buchanan *et al.*, 2017). The organism causes listeriosis when ingested in contaminated food (Bintsis, 2017). The organism can be killed by adequate cooking and pasteurization but in some Ready-to-Eat foods contamination can occur during the processing, during packaging or after the sterilization. They pose a health risk as they cannot be furtherly cooked to kill off contaminants before consumption (Bintsis, 2017).

2.11 Applications of Biosynthesized Silver Nanoparticles by Actinomycetes sp.

Biosynthesized silver nanoparticles from *Actinomycetes* sp. can be applied in different areas. They are mostly applied as biomedical therapy against pathogenic microorganisms. Actinomycetes have been exploited for their antibiotic producing ability and so is their biosynthesized nanoparticles. They have been found to be more toxic to pathogens than chemically produced silver nanoparticles (Abdeen *et al.*, 2014). Table 2.2 below shows various antimicrobial activities of biosynthesized silver nanoparticles from *Actinomycetes* sp.

Table 2.2: Activities of Biosynthesized Silver Nanoparticles by *Actinomycetes* Strains Against Pathogenic Bacteria

***Actinomycetes* sp. strain**	**Pathogenic bacteria**	**Main Result**	**Reference**
Streptomyces sp.	*S. aureus, E. coli, P. aeruginosa, K. pneumoniae, P. vulgaris.*	The nanoparticles had the highest antimicrobial activity against *P. aeruginosa* > *S.aureus* > *K.pneumonia*. The least were *E. coli and P. vulgaris*	Abdeen *et al.,* (2014).
Streptomyces sp.	*S. aureus, E. coli, P. vulgaris, Shigella dysentriae, Salmonella typhi and K. pneumoniae*	Silver nanoparticle showed antibacterial activity agaist all pathogens	Samainathan *et al.,* (2015)
Stretomyces sp., *Micromonospora* sp.	ESBL *E. coli*, ESBL *K. pneumoniae, E. faecium, S,aureus, P.aeruginosa, P.mirabilis, P. vulgaris*	ESBL *E. coli* and ESBL K. pneumoniae had the largest zone of inhibition.	Mohamed and Ahmed (2016)
Streptomyces sp.	*Escherichia coli* CBAM 0001, *Staphylococcus aureus* ATCC 25923, *Mycobacterium smegmatis* INCQS 061.	The synthesized nanoparticle was antagonistic against all pathogens with inhibition zones ranging from 12-34mm.	Silva-Vinhote *et al.,* (2017).
Nocardiopsis dassonvillei	*Staphylococcus aureus, Salmonella* sp., *Klebsiella pneumoniae, Pseudomonas*	The biosynthesized nanoparticles had the highest inhibition zone against K. pneumoniae at the lowest	Khalil *et al.,* (2022)

	aeruginosa, CoNs	nanoparticle
	Staphylococcus,	concentration 50µg/ml
	ESBL-prouducing	and at the highest
	Escherichia coli,	nanoparticle
	Proteus mirabilis	concentration 200µg/ml

CHAPTER THREE
METHODOLOGY

3.1 Materials

The materials used includes: Distilled water, Sterile petri dishes, Cotton wool, Aluminum foil, Alcohol (70% ethanol), Inoculating loop, Test tubes, Slides, Measuring cylinder, Conical flask, Beaker, Glass spreader, Media bottles, Micropipette, Micropipette tips, Dropper.

3.2 Culture Media

The culture media used includes: Nutrient agar, Nutrient broth, Methyl Red Vogues Proskauer broth, Mueller-Hinton Agar, Starch Agar, Starch Casein Agar for the isolation of actinomycetes.

3.3 Equipment and Reagent

Equipment used includes: Autoclave, Weighing balance, Water bath, Colony counter. Reagents used include: Gram's Iodine, Crystal violet, Methyl red, Safranin, naphthol solution, 40% KOH.

3.4 Sample Collection

The experiment was carried out on soil samples collected from the dumpsite in Mountain Top University. The samples were collected in sterile universal bottles and transported to the laboratory.

3.5 Preparation of Media

Nutrient agar and Starch Casein agar were used in this study. The media were prepared according to the manufacturers' instruction.

3.5.1 Nutrient Agar

The medium was prepared according to the manufacturer's instruction which stated that twenty-eight grams (28g) of the nutrient agar powder should be dissolved in one litre (1L) of distilled water. For this research, two point eight (2.8g) of nutrient agar powder was measured on a weighing balance into a sterile medium bottle. 100ml of distilled water was then added into the medium bottle. The bottle was covered tightly and swirled to dissolve the medium. The solution was then boiled in the water bath for proper homogenization after mixing. After boiling, the medium was autoclaved at 121^{o}C for 15 minutes. After autoclaving, the medium was allowed to cool to about 50^{o}C and then poured into sterile petri dishes after serial dilution

had been done. The media was swirled gently to ensure uniform spreading of the organisms in the plate. The agar plates were allowed to solidify and incubated.

3.5.2 Starch Casein Agar

This medium was primarily used to isolate *Actinomycetes* sp. For this experiment, 100 ml of the medium was used, simple mathematical calculations were used to measure the amount of the composing reagents. The medium was then boiled in the water bath for proper homogenization after which it was autoclaved at 121^{o}C for 15 minutes. The medium was then allowed to cool to about 50^{o}C then poured into sterile petri dishes after serial dilution had been carried out. The plates were gently swirled to ensure uniform spreading of the organisms in the plate.

3.6 Isolation of Actinomycetes

The Actinomycetes were isolated on Starch Casein Agar (SCA) using serial dilution. Glass test tubes containing 10 ml of distilled water, labelled 10^{-1} to 10^{-6} respectively. 1gm of soil sample was added to the first tube and swirled upside down few times. The serial dilution will be done from 10^{-2} to 10^{-6}. 0.1 ml aliquot of each dilution was pipetted into sterile petri dishes after which SCA which had cooled was poured onto the plate. The plates were incubated for 1-3 days at 37^{o}C. Colonies were selected and maintained on SCA plates (Bhosale *et al.*, 2015).

3.7 Pure Culture Technique

From the primary plates, different isolates were sub-cultured aseptically by streaking onto the prepared nutrient agar and starch casein agar plates. The plates were incubated for 24 to 48 hours at 37^{o}C. These resulted in pure culture of the isolated organisms. The pure culture isolated organisms were streaked onto prepared nutrient agar and starch casein agar slants and kept in the refrigerator.

3.8 Identification of Bacterial Isolates

The bacteria isolate from both starch casein agar and nutrient agar were identified using morphological appearance and biochemical tests (Sapkota *et al.*, 2020).

3.8.1 Morphological Identification

Morphological characterizations were done using colonial, cellular and pigment appearances on culture plates.

3.8.2 Biochemical Identification

Biochemical identification of isolates was done using Gram Staining, Catalase test, Starch hydrolysis test, Methyl red/ Voges Proskauer test, Simmons citrate test, Sugar fermentation test.

3.8.3 Gram Staining

Gram staining is essential for phenotypic characterization of bacteria. For the process, a smear of the organism was made on a glass slide using an inoculating loop and heat fixed. The primary stain which is crystal violet, was then applied unto the fixed smear for 60 seconds. After which, the stain is drained off the slide and rinsed with water. The mordant which is the Lugol's iodine was applied unto the smear for 60 seconds, after which it was drained off and rinsed with water. A few drops of ethyl alcohol which is the decolourizer is applied for 10 seconds and rinsed off with water. Lastly, the counter stain which is safranin was applied onto the smear for 60 seconds after which it was rinsed off. The slides were allowed to air dry. After drying, the slides were observed under the light microscope using the X100 lens. The aim of Gram staining was to determine the Gram reaction of the isolates, which could either be Gram-positive or Gram-negative bacteria (Bertrand Faurie, 2017).

3.8.4 Catalase Test

Catalase test is used to detect the production of the catalase enzyme in an organism. It differentiates between catalase-producing organisms (Staphylococci and Micrococci) and organisms that do not produce catalase (Streptococci). The slide method was used. A smear of the organisms was made on clean slides and a drop of hydrogen peroxide was added to each smear. Each slide was observed for formation of air bubbles which indicated a positive result (Cheesebrough, 2000)

3.8.5 Methyl Red/ Voges Proskauer (MRVP) Test

The MRVP broth was prepared according to manufacturer's instruction. 2.55 g of MR-VP broth powder was dispensed into a clean conical flask, into which 150 ml of distilled water was added. The mixture was homogenized gently to dissolve completely. 10 ml of the broth was dispensed into each test tube, covered tightly with corks and sterilized in an autoclave for 15 minutes at 121^{o}C. After sterilization, the broth was allowed to cool, then each isolate was inoculated into each tube with proper labelling. The tubes were incubated at 37^{o}C for 24 hours. Afterwards, 1ml of each broth was dispensed into clean test tubes for the VP test. 5 drops of methyl red reagent was added to each broth. The appearance of a red colour indicates a positive

reaction while the appearance of a yellow colour indicates a negative reaction. For the VP test, Barritt's method was used. 0.5 ml of 6% α-naphthol solution and 0.5ml of KOH was added to the 1ml broth tubes and the tubes were shaken. The development of a pink colour after 30 minutes indicates a positive reaction (Olutiola *et al.,* 2000).

3.8.6 Starch Hydrolysis Test

The starch agar which consists of nutrient agar and 1% starch is prepared and sterilized. 20 ml of the molten starch agar is poured into sterile petri dishes and allowed to solidify. The isolates are inoculated on the surface of the starch agar plates and incubated at 37^{o}C for 24-48 hours. Afterwards the plates were flooded with some quantity of Iodine (Olutiola *et al.,* 2000).

3.8.7 Citrate Utilization Test

This test is used to detect the ability of an organism to use sodium citrate as the only source of carbon and inorganic ammonium salts as the only nitrogen source. The medium used was Simmons Citrate Agar. The medium was prepared according to manufacturer's instruction, 3.64 g of the agar powder was measured into a clean conical flask and 150 ml of distilled water was measured into the flask which was gently swirled to completely dissolve the medium. 10ml of the medium was dispensed into test tubes and corked properly. The test tubes were sterilized in an autoclave for 15 minutes at 121^{o}C. After sterilization, the tubes were allowed to cool in a slanting position. The tubes were properly labelled for each isolate and the tubes were then inoculated with the isolates. The corks were loosely fitted and the tubes were placed in an incubator at 37^{o}C for 18-24 hours. The development of a blue colour along the line of the growth indicated a positive result (Olutiola *et al.,* 2000).

3.8.8 Sugar Fermentation Test

This test is to determine the ability of microorganisms to ferment carbohydrates and produce organic acid and gas as end products. The medium used was prepared using 1% of peptone, 1% of the fermentable sugar 0.1% of sodium chloride and 0.0025% of bromocresol purple which served as the indicator and the appropriate amount of distilled water. 10ml was dispensed into each test tube with Durham tubes dropped upside down into the test tubes and corked appropriately. The medium was sterilized at 121^{o}C for 15 minutes, after which the isolates were inoculated into each tube with proper labelling. The tubes were incubated for 18-24 hours at 37^{o}C. Isolates which fermented the sugars produced acid, turning the medium yellow, fully or partially. Gas production was indicated by the presence of gas bubbles in the Durham tubes.

Some isolates fermented the sugars fully with acid and gas production while others produced no gas or did not ferment the sugar at all (Olutiola *et al.,* 2000)

3.9 Screening of Bacterial Isolates for Antibiotics Potentials

The antibiotic effect of the isolates was tested using agar well diffusion method. The isolates were grown and maintained in nutrient broth for 24-48 hours. The medium used was Mueller-Hinton Agar, 7 g of Mueller-Hinton agar powder was weighed into a clean conical flask and 200ml of distilled water was measured into the flask. The mixture was sterilized for 15 minutes at 121^{o}C. After sterilization, the medium was allowed to cool to about 50^{o}C then aseptically poured into sterile petri dishes. The agar plates were allowed to solidify, then sterile 1ml pipette tips were used to aseptically bore holes in the agar plates, which served as the wells. After it solidified, the pathogenic *Escherichia coli* was inoculated onto the surface of the agar using a loop full of the broth culture. 0.1ml of the broth cultures were dispensed into the wells, with proper labelling. The plates were incubated for 24 hours in an upright position. After incubation, the presence of zones of inhibition around the well indicated antibacterial activity, the diameter of the zones was measured and recorded.

3.10 Synthesis of Bioactive Silver Nanoparticles

The isolates with the highest zones of inhibition were grown in nutrient broth for 72 hours in an incubator at 37^{o}C. The silver nitrate was prepared using silver nitrate crystals and distilled water. 250 ml of 10 mM silver nitrate solution was used to prepare the synthesize the silver nanoparticles. The 250 ml silver nitrate solution was added into 250 ml of broth culture. The flasks were incubated at 37^{o}C for 72 hours in dark condition, in a shaking incubator and observed for dark brown colour change which indicates nanoparticles synthesis (Bhosale *et al.,* 2015).

3.11 Characterization of Nanoparticles

The synthesized nanoparticles were characterized using UV-VIS Spectroscopy and FTIR analysis (Bhosale *et al.,* 2015).

3.11.1 UV-Vis Spectroscopic Analysis

Silver nanoparticles synthesized by both isolates were analysed at periodic intervals at a wavelength of 200-800 nm (Bhosale *et al.,* 2018). The synthesis was confirmed by the peaks observed in the UV-vis graphs of the reaction.

3.11.2 Fourier Transform Infrared Spectroscopy (FTIR)

The silver nanoparticles were also probed for the presence of biomolecules that carry out the bio-reduction of silver ions in the synthesis process and the stabilization of silver nanoparticles within the suspension (Silva-Vinhote *et al.,* 2017).

3.12 Antibiotic Effect of Silver Nanoparticles against Pathogens

The antibiotic effect of the synthesized silver nanoparticles was determined against the following pathogens: *Escherichia coli, Staphylococcus aureus, Salmonella enterica, Proteus* sp. and *Enterococcus faecalis,* using the agar well diffusion methods. The isolates were grown and maintained in broth cultures. The medium used was Mueller-Hinton Agar which was prepared according to manufacturer's instruction. 7.2g of Mueller-Hinton agar powder was weighed into a clean conical flask and 200ml of distilled water was measured into the flask. The mixture was swirled gently to dissolve the powder and corked. The mixture was autoclaved for 15 minutes at 121^{o}C. After sterilization, the medium was allowed to cool to about 50^{o}C then aseptically poured into sterile petri dishes. The agar plates were allowed to solidify, then sterile 1ml pipette tips were used to aseptically bore four holes in each agar plates, which served as the wells. Molten Mueller-Hinton agar was then used to seal the bottom of the holes to avoid growth at the bottom of the plates. The pathogenic isolates were then streaked across the surface of the agar plates with proper labelling. The biosynthesized silver nanoparticle, the silver nitrate solution, and antibiotic solution and the broth culture were aseptically dispensed into each of the wells. The plates were incubated in an upright position for 18-24 hours. After incubation, the plates were observed for zones of inhibition which were measured and recorded.

CHAPTER FOUR
RESULTS AND DISCUSSION

4.1 Colony Count

Thirteen (13) morphologically different bacteria were isolated from the dumpsite located in the University premises. The isolation process was carried out twice from the same location. Table 4.1 shows the colony counts for the first and second isolations respectively in each of the serial dilutions investigated. The colonies after 24 hours incubations ranged from 218-924 x $10^4$and 20-100 x10^6 cfu/ml respectively for both sampling intervals. The highest colony count was observed at 924 x 10^{-4} and 100 x 10^{-6} cfu/ml. The prevalence of bacterial species in the dumpsite soil is due to the high levels of liquid effluents containing heavy metals (Olanrewaju, 2002). Refuse dumpsites also serve as a rich source of nutrients to diverse microorganisms (Odeyemi *et al.*, 2011). Odeyemi, (2012), isolated bacterial species from 3 sites around a dumpsite and from the dumpsite itself in Ekiti state, Nigeria. Sampling was done over a five (5) day period at all four (4) sites. The dumpsite had the highest colony count for day three and four, while the sampling site 150 m away from the dumpsite had the highest colony counts on day one, two and five. This is synchronous with the findings by Odeyemi *et al.* (2011) that dumpsites are rich sources of microorganisms.

4.2 Morphological Characterization of the Isolates

Table 4.2 shows the morphological characteristics of the isolates including their colour, shape, surface, edge and elevation. The observed colours include: cream, white, milky, yellow and dark green. The shapes include: irregular, circular, rhizoid and punctiform. The elevation include: raised, umbonate, crateriform and convex. The observed edges include: lobate, entire and undulate. In a similar research work by Prakash *et al*. (2014), two species of *Actinomycetes* were isolated from soil sample, *Streptomyces* sp. and *Streptoverticillium* sp. Both had white coloured aerial mass with linear chained spores. On the reverse side of the plate, *Streptomyces* sp. exhibited a coral pink to red pigmentation while *Streptoverticillium* sp. exhibited no pigmentation. Under the microscope, *Streptomyces* sp. showed a coiled or hook like structure, while *Streptoverticillium* sp. showed a V shaped linear spore chain at the end of the mycelium. Mahmoud *et al*. (2016) isolated *Streptomyces* sp. from soil samples. They showed morphological characteristics such as: spiral and looped spore chain, smooth and spiny spore surface, grey and greenish-grey aerial mycelium colour and brownish substrate mycelium. Shreshtha *et al*. (2021) isolates *Actinomyces* sp. from soil sediments in Nepal on Starch Casein Agar plates, which exhibited light yellow to orange-red colonies, blue green colonies, white grey to pinkish colonies and dark brown to black spore colonies. The colonies appeared to be waxy, shiny, powdery with concave and convex elevations.

Table 4.1: Colony Counts for Soil Samples

Sampling Location	Sampling interval	Colony counts/ Serial dilution factors/	
		At 10^{-4} (cfu/ml)	**At 10^{-6} (cfu/ml)**
Hostel dump site	First sampling	924×10^4	20×10^6
	Second sampling	218×10^4	100×10^6

Table 4.2: Morphological characteristics of the isolates

Isolates	Colour on Nutrient Agar	Shape	Elevation	Surface
MDN1	White	Irregular	Crateriform	Rough
MDN2	White	Rhizoid	Crateriform	Dull
MDN3	Cream	Circular	Raised	Smooth
MDN4	White	Irregular	Convex	Dull
MDN 5	Milky	Circular	Raised	Rough
MDN 6	Milky	Circular	Raised	Dull
MDN 7	Milky	Irregular	Raised	Glistening
MDN 8	Milky	Irregular	Crateriform	Dull
MDN 9	Cream	Irregular	Convex	Rough
MDC 1	Dark green	-	-	Rough
MDC 2	White	Circular	Crateriform	Rough
MDC 3	Dark green	-	-	Rough
MDC 4	Dark green	-	-	Rough

4.3 Biochemical Characterization of the Isolates

Table 4.3 shows the biochemical characteristics of the isolates including: Gram staining, MR-VP tests, starch hydrolysis tests, catalase tests, sugar fermentation tests (glucose and galactose) and citrate utilization tests. Both positive and negative reactions to the test reagents were observed. The probable microorganisms isolated includes: *Bacillus* sp., *Clostridium* sp., *Staphylococcus* sp., *Actinomycetes* sp. and *Listeria* sp. In a similar research, Shreshtha *et al*., (2021), isolated *Actinomycetes* sp. on Starch Casein Agar which were Gram positive, non-acid fast coccoid and bacillary cells. In contrast to this study, Samainathan (2015), screened their *Streptomyces* sp. isolates using Nitrate reductase test, where a pinkish colouration indicates a positive test. Similarly, Mahmoud *et al*., (2016) also used nitrate reductase enzyme test to screen their isolates for Actinomycetes, where two were found to be positive for *Actinomycetes* species.

Table 4.3 Biochemical Characterization of the Isolates

Isolates	Gram Staining	Shape	Arrangement	Catalase	Methyl Red	VP Test	Starch	Citrate	Glucose		Galactose		Sucrose		Fructose		Probable Isolates
									Acid Production	Gas Production	Acid Production	Gas Production	Acid Production	Gas Production	Acid Production	Gas Production	
MDN 1	+	Cocci	Cluster	+	-	-	+	+	+	+	+	+	+	+	+	+	*Staphylococcus* sp.
MDN 2	+	Bacilli	Clustered	+	+	+	-	+	+	-	+	-	+	-	+	+	*Listeria* sp.
MDN 3	+	Cocci	Cluster	+	-	+	-	+	+	-	-	-	-	+	+	-	*Staphylococcus* sp.
MDN 4	+	Bacilli	Diplobacilli	+	+	+	-	+	+	-	-	-	+	+	-	-	*Clostridium* sp.
MDN 5	+	Cocci	Clustered	+	-	-	-	+	+	+	+	+	+	+	+	+	*Staphylococcus* sp.
MDN 6	+	Bacilli	Singly	+	-	-	+	+	+	-	+	-	+	-	+	+	*Bacillus* sp.
MDN 7	+	Bacilli	Clustered	-	-	+	+	+	+	-	-	-	+	-	+	+	*Staphylobacillus* sp.
MDN 8	+	Bacilli	Clustered	-	-	+	+	+	+	-	-	-	+	+	+	+	*Actinomycetes* sp.
MDN 9	+	Bacilli	Singly	+	+	+	+	+	+	-	-	-	+	-	+	-	*Actinomycetes* sp.
MDC 1	+	Bacilli	Clustered	+	+	+	-	+	-	-	-	-	-	-	-	-	*Actinomycetes* sp.
MDC 2	+	Bacilli	Clustered with filaments	-	+	+	-	+	+	+	-	-	+	-	+	+	*Actinomycetes* sp.
MDC 3	+	Bacilli	Clustered with spores and sporangia	-	+	-	-	+	+	-	+	-	-	-	-	+	*Actinomycetes* sp.
MDC 4	+	Bacilli	Clustered with spores	+	+	+	-	+	+	+	+	+	+	+	+	+	*Actinomycetes* sp.

4.4 Antibacterial Activity of the Isolates

Table 4.4 shows the measured zones of inhibition of the isolates tested against pathogenic *Escherichia coli* using agar well diffusion method. Some isolates show no zone of inhibition while some showed significant zones of inhibition. MDN9 showed the highest zone of inihibition at 55 mm followed by MDC4 which showed a zone of 50 mm diameter. Isolates such as MDN1, MDN4, MDN6, MDN7, MDN8 and MDC2 showed no zone of inhibition against the pathogen. Similarly, Velayudham and Murugham (2012) isolated thirty-six (36) species of *Actinomycetes* sp. from forest soil. The antimicrobial activity of these isolates were tested against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumonia* and *Candida albicans*. The zones of inhibition ranged between 24 mm and 0 mm for *E.coli*, 18 mm and 0 mm for *B. subtilis*, 18 mm and 0 mm for *S. aureus*, 21 mm and 0 mm for *P. aeruginosa*, 21 mm and 0 mm for *C. albicans*. Silva-vinhote *et al.*, (2017), isolated three (3) species of *Actinomycetes* sp. from Amazonic soil and their antimicrobial activity was tested against some known pathogens such as: *Escherichia coli, Staphylococcus aureus, Mycobacterium smegmatis*, *Candida albicans* and *Candida valderwaltii.* The zones of inhibition ranged from 20 mm to 13 mm for *E. coli*, 34 mm to 23 mm for *S. aureus*, 27 mm to 0 mm for *M. smegmatis*.

Table 4.4: Antibacterial Activity of the Isolates

Isolates	Diameter of Zone of Inhibition (mm)
MDN 1	nz
MDN 2	20
MDN 3	30
MDN 4	nz
MDN 5	33
MDN 6	nz
MDN 7	nz
MDN 8	nz
MDN 9	55
MDC 1	36
MDC 2	nz
MDC 3	34
MDC 4	50

Key: nz- No Zone

4.5 Synthesis of Nanoparticles

Two of the isolates with the highest zones of inhibitions were used in synthesizing silver nanoparticles. The broth cultures began to show brown colouration after 24 hours of incubation. After 72 hours, both broth cultures had turned dark brown from the light-yellow broth colour before incubation. Figure 4.1 and 4.2 below shows the silver nitrate solution, the 72 hours broth culture and the synthesized nanoparticle with the dark brown colouration. The evident colour change is due to excitation of surface plasmon vibrations in the silver nanoparticles (Mulvaney, 1996). The appearance of a dark-brown colouration in the biomass has been reported to indicate the bio-reduction of the silver ions and formation of silver nanoparticles (Verma and Mehata, 2016; Mahmoud *et al*. 2016). Silver nanoparticles have been synthesized successfully from other sources such as plant roots, stems, microorganisms such as *Bacillus* sp., *Pseudomonas* sp. (Elbeshehy *et al.,* 2015; Lyudmila *et al*., 2018; Wan Mat Khalir *et al.,* 2020; Khanal *et al.*, 2022). As observed in this study. the colour change from light yellow to light brown after 24 hours of incubation indicated the bio-reduction of the silver ions. After 72 hours, the observed dark-brown colouration indicated synthesis of silver nanoparticles. This is in contrast to the study carried out by Silva-Vinhote *et al*, (2017), who reported a light-yellow colouration for the biosynthesized silver nanoparticles from *Strptomyces* sp. DPUA 1747 under the same duration of incubation. Similarly, Samainathan (2015) synthesized silver nanoparticles using soil *Streptomyces* sp. and 1mM silver nitrate solution, the synthesis was confirmed by the colour change to yellow. Bhosale *et al.* (2015) synthesized silver nanoparticles using soil sourced *Actinomycetes* sp. and silver nitrate solution, resulting in a colour change from yellow to brown.

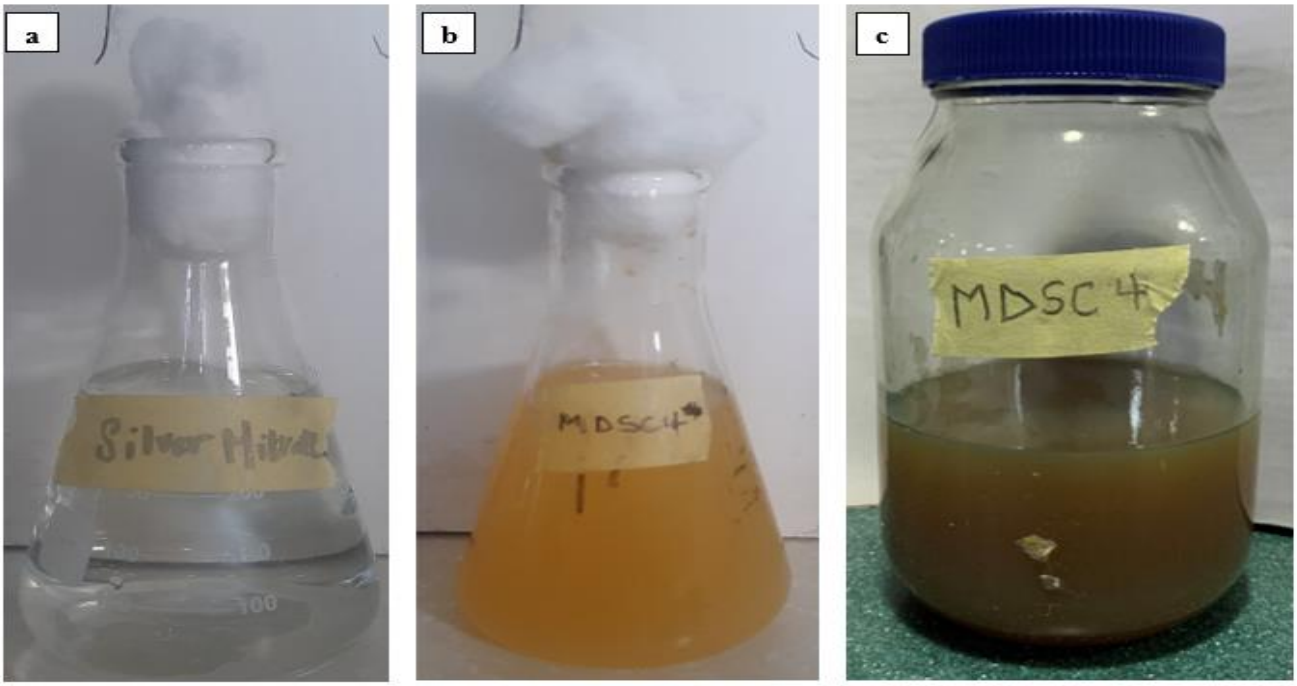

Figure 4.1: Visual Observation of Biosynthesized Silver NPs by isolate MDSC4 (a) Silver nitrate solution (b) Broth culture (c) Synthesized silver nanoparticles

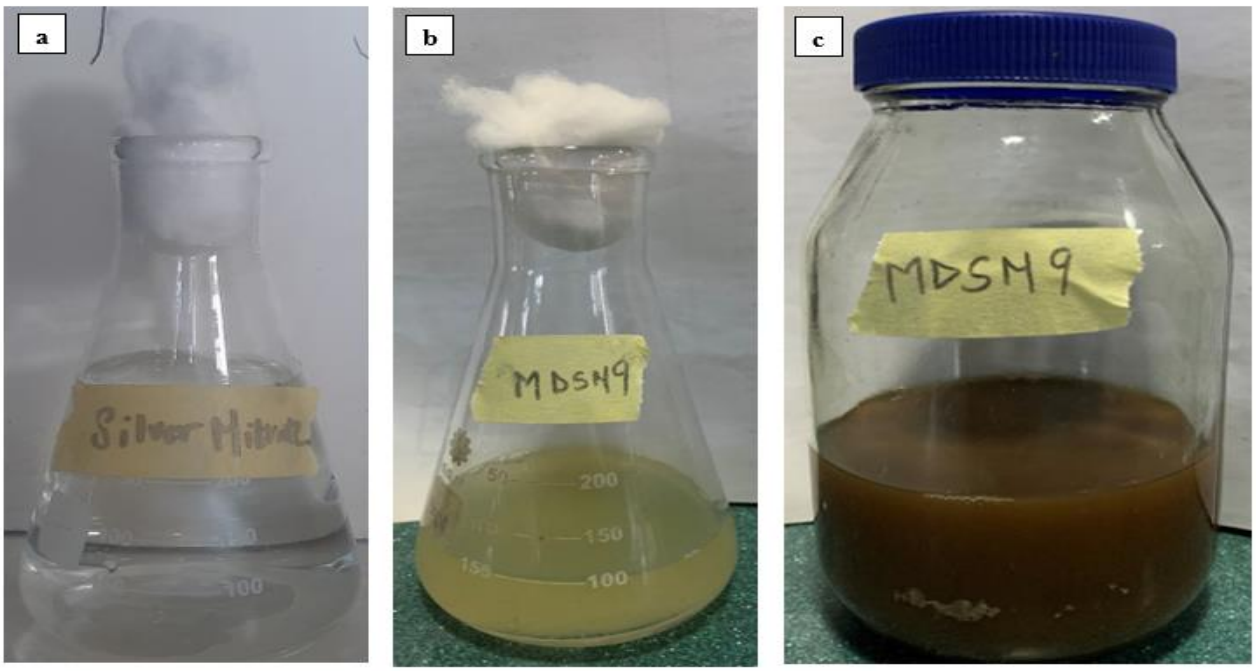

Figure 4.2: Visual Observation of Biosynthesized Silver NPs by isolate MDSN9 (a) Silver nitrate solution (b) Broth culture (c) Synthesized silver nanoparticles.

4.6 Characterization of Nanoparticles

The biosynthesized silver nanoparticles were further taken for further analysis to determine the significant properties using UV-Visible spectroscopy and Fourier Transform Infrared Spectroscopy (FTIR). The use of UV-Visible spectroscopy and FTIR spectroscopy has been reported as efficient means of characterizing silver nanoparticles (Agustina *et al.*, 2021; Alharbi *et al.*, 2022). Silva-Vinhote *et al.* (2017) characterized the synthesized silver nanoparticles produced from amazonic biome using UV-Visible spectroscopy, FTIR, X-ray Diffusion (XRD) and Nanoparticle Tracking Analysis (NTA). Similarly, Bhosale et al., (2015) characterized the silver nanoparticles by UV-Vis spectroscopy, FTIR analysis, Scanning Electron Microscope (SEM) analysis.

4.6.1 UV- Visible Spectroscopy

The bio-reduction of the silver ions was monitored at 24 hours intervals for 3 days by using a UV-visible spectrophotometer. The values were taken between wavelengths of 200-800 nm. Figure 4.3 and 4.4 below show the UV- visible spectra for MDSC4 and MDSN9 synthesized silver nanoparticles. In Fig. 4.3, the silver nanoparticles had maximum absorbance at 350 nm after 48 hours and a maximum absorbance at 400 nm after 72 hours. In Fig. 4.4 below, an absorbance peak was observed at about 400 nm after 72 hours. Similar studies by El Nagar and Abdelwahed, (2013) and Agustina *et al*. (2021) on the synthesis and characterization of silver nanoparticles showed a broad peak at 400 nm which is synchronous with the Surface Plasmon Resonance (SPR) of silver nanoparticles. In contrast, Sukanya *et al*., (2013) synthesized silver nanoparticles from *Streptomyces* sp. with intense absorption peak at 450 nm. In another study by Priyaragini *et al.* (2013), it was reported that silver nanoparticles biosynthesized by actinobacteria with sharp narrow absorption peak located between 420-450 nm. It has been reported that the dark brown colouration exhibited by silver nanoparticles is due to excitation of surface plasmon vibrations in the metal nanoparticles (Tenderwealth *et al*., 2018).

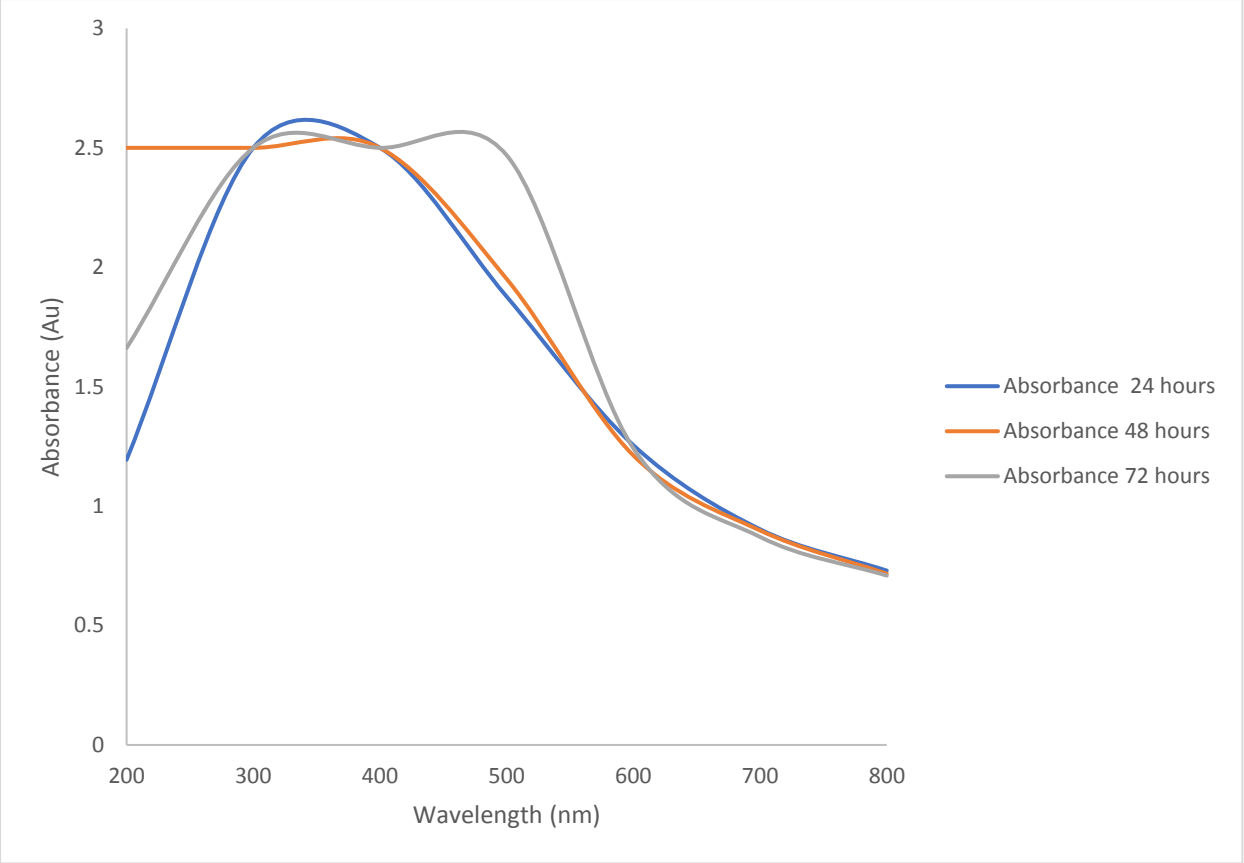

Figure 4.3: UV-visible spectrum of the Biosynthesized Silver Nanoparticles by MDN 9 at Different Incubation Periods

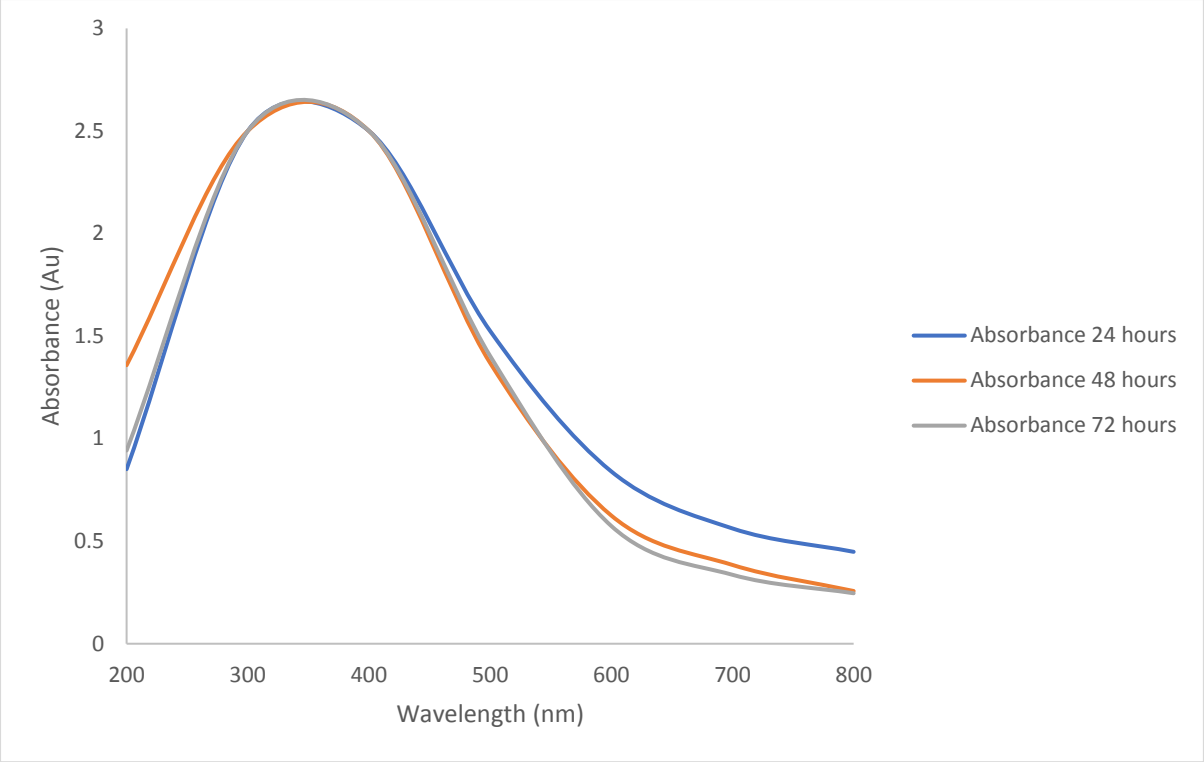

Figure 4.4: UV-visible spectrum of the Biosynthesized Silver Nanoparticles by MDN 9 at Different Incubation Periods

4.6.2 Fourier Transformation Infrared Spectroscopy (FTIR)

The Fourier transformation infrared spectroscopy (FTIR) spectrum of the greenly synthesized *Actinomycetes* sp. -AgNPs is shown in Figure 4.5 and 4.6 below. 15 peaks were present between 3762 cm^{-1} to 360 cm^{-1}. The FTIR spectrum showed a broad peak at 3438 cm^{-1} which corresponds to the broad intermolecular bonding of the alcoholic OH band. The peak at 2926.29 cm^{-1} indicates an alkane C-H stretching vibration. The peak at 1728.45 cm^{-1} indicates the strong aldehyde C=O stretching. The peak at 1637.57 cm^{-1} shows the medium C=C stretching of the alkene group. Furthermore, the peak at 1514.38 cm^{-1} indicates a strong nitro compound group from the N-O stretching. The peak at 1381.79 cm^{-1} indicates medium aldehyde C-H bending. The peak at 1329.00 cm^{-1} shows the presence of alcohol group indicating the medium O-H bending. The peak at 1256.29 cm^{-1} indicates the strong aromatic ester bond of the C-0 stretching. The presence of a strong sulfoxide group with S=O stretching is indicated in the 1039.46 cm^{-1} peak and a peak at 605.00 cm^{-1} indicates the presence of a halo compound with C-I stretching. This organic groups are present due to the biological reduction of silver ions which synthesized the silver nanoparticles (Samainathan, 2015). In similar research by Bhosale *et al.* (2015), the FTIR analysis showed peaks of absorption at 1636.3 cm^{-1} and 3358 cm^{-1}. The peak at 1636.3 cm^{-1} is characteristic of -C=O carbonyl groups and -C=C stretching, while the peak at 3358 cm^{-1} showed the stretching of bonded hydroxyl (-OH) group and H-bonded. The FTIR analysis of the silver nanoparticles synthesized by Samainathan (2015), showed absorbance peaks at 600 cm^{-1} and 4000cm^{-1}. Spectral bands were prominent at 1137 cm^{-1}, 1165 cm^{-1} and 3483 cm^{-1}.

Table 4.5: F-T-IR spectral positions with their corresponding vibration modes for biomass filtrate and Ag-NPs synthesized by *Actinomycetes* sp. -AgNPs

Absorption frequency (cm^{-1})	Chemical groups	Compound class
3762.00	-	-
3438.00	O-H stretching	Alcohol
2926.29	C-H stretching	Alkane
2369.33	-	-
1728.45	C=O stretching	Aldehyde
1637.57	C=C stretching	Alkene
1514.38	N-O stretching	Nitro compound
1427.00	-	-
1381.79	C-H bending	Aldehyde
1329.00	C-H bending	Alcohol
1256.29	C-O stretching	Aromatic ester
1039.46	S=O stretching	Sulfoxide
605.00	C-I stretching	Halo compound
459.76	-	-
360.52	-	-

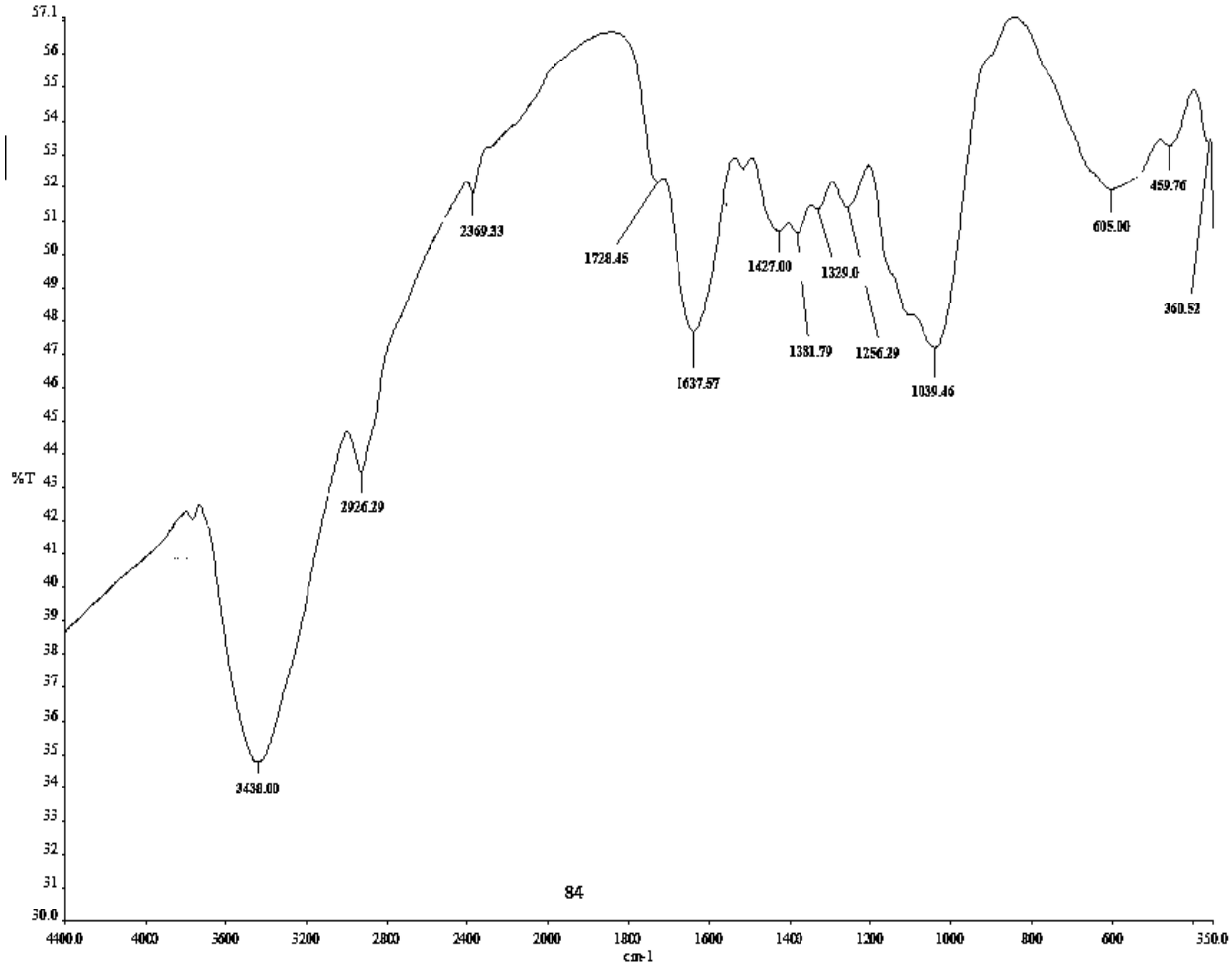

Figure 4.5: Fourier Transform Infrared (FTIR) Spectrum of the Biosynthesized Silver Nanoparticles by MDC 4 at Different Incubation Periods.

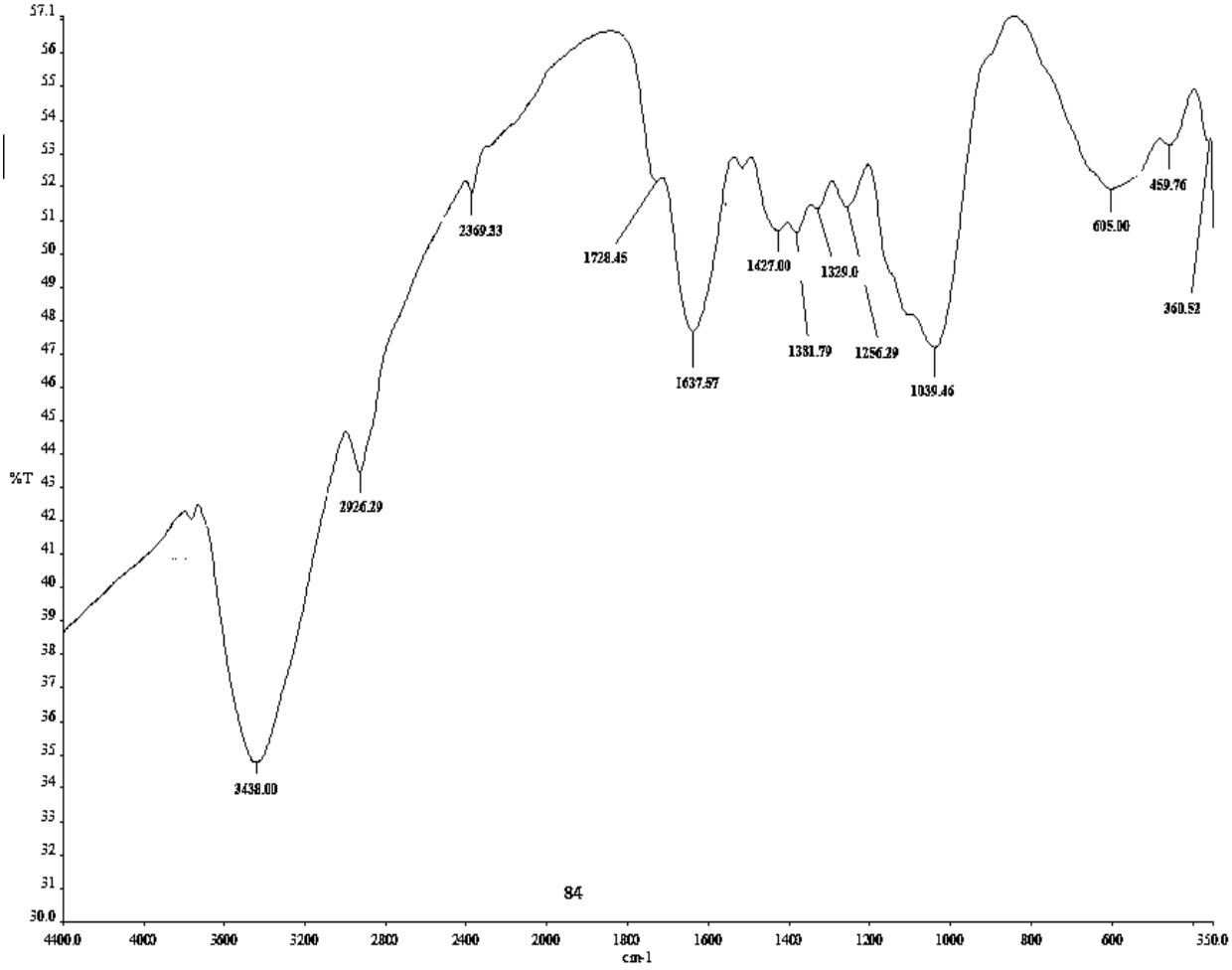

Figure 4.6: Fourier Transform Infrared (FTIR) Spectrum of the Biosynthesized Silver Nanoparticles by MDN 9 at Different Incubation Periods.

4.7 Antimicrobial Activity of Biosynthesized Silver Nanoparticles

The antimicrobial activity of the greenly synthesized silver nanoparticles was investigated against selected known bacterial pathogens. The nanoparticles showed significant effectiveness against different pathogenic bacteria. The highest inhibition zone was observed against *Proteus* sp. and *E. coli*. The inhibition zones varied from 16-27 mm and 12-45 mm for MDC4 and MDN9 respectively. Some pathogens showed no zones of inhibition. Table 4.5 and 4.6 below shows the observed inhibition zones against the silver nanoparticles, silver nitrate, chloramphenicol and the bacterial extract.

The antimicrobial property of the synthesized silver nanoparticles was determined against five pathogenic microorganisms. The silver nanoparticles synthesized from isolate MDC4 showed the largest zone of inhibition against *Proteus* sp. (27 mm)., followed in order by *E. coli* which showed a zone of 19 mm, while *Enterococcus faecalis* showed the least zone of inhibition. On the other hand, silver nanoparticles biosynthesized from MDN9 inhibited *E. coli* and *Salmonella enterica* best with zones of 45 mm and 15 mm respectively compared to *Proteus* sp. and E. *faecalis* which showed no zones of inhibition. The silver nanoparticles yielded the largest inhibition zone. In similar research by Sukanya *et al.*, (2013), the biosynthesized silver nanoparticles with the characteristic brown pigmentation were tested against various pathogens including Gram-positive *S. aureus* and Gram-negative *P. mirabilis, E. coli, K. pneumoniae, P. aeruginosa and P. vulgaris*. The nanoparticles had the highest activity against *P. aeruginosa,* followed in order by *K. pneumoniae* and the least for *E. coli.* This result was compared with the commercially produced kanamycin antibiotic which was not as efficient as the silver nanoparticles in inhibiting the above pathogens. Bhosale *et al.* (2015) carried out similar research on the "Biosynthesis, characterization and study of antimicrobial effect of silver nanoparticles by *Actinomycetes* spp". The efficiency of the synthesized silver nanoparticles were tested for antimicrobial activity against *E. coli, K. pneumoniae* and *P. aeruginosa,* and also compared with regular antibiotics, and silver nitrate. The silver nanoparticles showed better antimicrobial property than commercially produced antibiotics but not as effective when compared combined with the antibiotics. The silver nitrate solution had the lowest antimicrobial activity against the pathogens.

Table 4.6: Antimicrobial Activity of Biosynthesized AgNPs from MDC4

Pathogens	Zones of Inhibition (nm)			
	Bacterial Extract	**Biosynthesized AgNPs**	**Silver Nitrate ($AgNO_3$)**	**Chloramphenicol**
Escherichia coli	11	19	13	16
***Proteus* sp.**	nz	27	nz	nz
Staphylococcus aureus	11	14	11	13
Enterococcus faecalis	nz	13	nz	nz
Salmonella enterica	nz	17	15	16

nz- No zone

Table 4.7: Antimicrobial Activity of Biosynthesized AgNPs from MDN9

Pathogens	Zones of Inhibition (nm)			
	Bacterial Extract	**Biosynthesized AgNPs**	**Silver Nitrate ($AgNO_3$)**	**Chloramphenicol**
Escherichia coli	30	45	12	12
***Proteus* sp.**	nz	Nz	nz	nz
Staphylococcus aureus	nz	12	14	nz
Enterococcus faecalis	nz	Nz	nz	nz
Salmonella enterica	nz	15	12	nz

nz - No zone

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The soil assessed in this study is a rich habitat that harbors diverse microorganisms including antimicrobial producing bacteria. Out of the 13 bacteria isolated in this study, isolate MDN9 and MDC4 identified using microscopic and biochemical characteristics as *Actinomycetes* sp. showed the highest zone of inhibition against the test pathogens, and was selected for further studies. *Actinomycetes* sp. MDN9 and MDC4 were able to reduce $AgNO_3$ for nanoparticle biosynthesis. The synthesized nanoparticles were characterized using UV-Visible Spectrophotometer and Fourier transform infrared spectroscopy (FT-IR) which confirmed the synthesis of nanoparticle. The biosynthesized silver nanoparticles (AgNPs) showed improved inhibitory activities against the five selected pathogenic bacteria including *S. enterica*, *S. aureus*, *E. coli*, *E. faecalis*, and *P. mirabilis* compared to the bacterial extract, and synthetic chloramphenicol antibiotics. Thus, this study revealed that AgNPs is a good antimicrobial agent that can be commercially developed as antibiotics against common infectious bacteria. s

5.2 Recommendation

Based on the research carried out, the following recommendations were made:

- *Actinomycetes* sp. with antimicrobial activity should be use in synthesizing metal nanoparticles.
- Biosynthesized silver nanoparticles from *Actinomycetes* sp. should be used in the industrial production of antibiotics.
- Strain improvement can be conducted on *Actinomycetes* sp. to improve the antimicrobial activity and nanoparticle production.

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APPENDIX 1

RAW DATA

UV-VIS Spectrometry for Silver Nanoparticles- MDN 9

Wavelength (nm)	Absorbance (24 hrs)	Absorbance (48 hrs)	Absorbance (72 hrs)
200	1.194	2.500	1.662
300	2.500	2.500	2.500
400	2.500	2.500	2.500
500	1.877	1.951	2.469
600	1.255	1.212	1.242
700	0.903	0.898	0.871
800	0.730	0.717	0.709

UV-Visible Spectrometry for Silver nanoparticles -MDC 4

Wavelength (nm)	Absorbance (24 hrs)	Absorbance (48 hrs)	Absorbance (72 hrs)
200	0.851	1.358	0.942
300	2.500	2.500	2.500
400	2.500	2.500	2.500
500	1.525	1.369	1.403
600	0.840	0.625	0.574
700	0.562	0.384	0.336
800	0.448	0.257	0.246

APPENDIX 2

COMPOSITION OF AGAR USED

Composition of Nutrient Agar

Composition	Amount
Peptone	5 g
Yeast Extract	2 g
Beef Extract	1 g
Sodium Chloride	5 g
Agar	15 g
Distilled Water	1 L

Composition of Starch Casein Agar

Composition	Amount
Sodium Chloride	2 g
Calcium Carbonate	0.02 g
Iron (II) Sulphate (heptahydrate)	0.01 g
Agar	18 g
Soluble Starch	10 g
Casein	0.3 g
Potassium nitrate	2 g
Magnesium Sulphate (heptahydrate)	0.05 g
Dipotassium hydrogen phosphate	2 g
Distilled Water	1 L

Composition of Simmons Citrate Agar

Composition	Amount
Sodium Chloride (NaCl)	5.0 g
Sodium Citrate (dehydrate)	2.0 g
Ammonium Dihydrogen Phosphate	1.0 g
Dipotassium Phosphate	1.0 g
Magnesium Sulfate (heptahydrate)	0.2 g
Bromothymol Blue	0.08 g
Agar	15 g
Distilled water	1 L

Composition of Starch Agar

Composition	Amount
Peptone	5 g
Yeast Extract	2 g
Beef Extract	1 g
Sodium Chloride	5 g
Agar	15 g
Distilled Water	1 L
Starch	1%