T.R.
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DEPARTMENT OF NANOSCIENCE AND NANOTECHNOLOGY

# BIOACTIVITY CHARACTERISTICS OF SILVER NANOPARTICLES SYNTHESIZED BY *STREPTOMYCES* SP. BSP1

Master's Thesis

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Supervisor
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# ÖZET

## *STREPTOMYCES* SP. BSP1 TARAFINDAN SENTEZLENEN GÜMÜŞ NANOPARTİKÜLLERİNİN BİYOAKTİVİTE ÖZELLİKERİ

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*Actinobacteria* üyeleri, çeşitli biyoaktif metabolitleri üretmelerinden dolayı en üretken mikrobiyal gruplardan biri olarak kabul edilmektedir. Özellikle endofitik aktinobakteriler, indirgeme aktivitesi ile nanopartikül sentezini kolaylaştıran proteinleri, enzimleri ve sekonder metabolitleri yüksek düzeyde salgılamaları ile karakterize edilmektedir. Bu tez çalışmasında, daha önce yapılan bir çalışmada bir baklagil nodülünden izole edilmiş olan *Streptomyces* sp. BSP1 kullanılarak gümüş nanopartikülleri sentezlenmiştir. Sentezlenen gümüş nanopartikülleri UV-vis spektroskopi, FTIR, XRD, SEM ve EDX analizleri ile doğrulanmıştır. UV-vis spektrum analizinde 439 nm'de maksimum absorbans gözlenmiştir. SEM analizi, sentezlenen nanopartiküllerin düzenli küresel biçimde ve ortalama 7.7-15.3 nm boyutta olduğunu göstermiştir. XRD analizi, sentezlenen gümüş nanopartiküllerinin kristal yapıda olduğunu ortaya çıkarmıştır. Ayrıca, *Streptomyces* sp. BSP1'in tüm genom dizisi NCBI GenBank'tan indirilerek kapsamlı genom analizi yapılmıştır. *Streptomyces* sp. BSP1'in genom dizisi RAST sunucusunda (https://rast.nmpdr.org/rast.cgi) annote edilmiş ve genom büyüklüğünün 7.4 Mb, GC içeriğinin %73.1 olduğu tespit edilmiştir. Genomda protein kodlayan 6868, RNA kodlayan 76 dizi bulunmaktadır. Filogenetik açıdan *Streptomyces* sp. BSP1'in karşılaştırmalı genom analizi TYGS sunucusunda (https://tygs.dsmz.de/) gerçekleştirilmiştir. *Streptomyces* sp. BSP1'in *Streptomyces albidoflavus* NRRL B-1271 ile yakın ilişkili olduğu ve bu tür ile en yüksek DNA-DNA hibridizasyon değerinin %65.2 olduğu tespit edilmiş olup bu değer genomik türleri sınırlandırmak için kullanılan %70 değerinden düşüktür. Ayrıca, sekonder metabolit kodlayan biyosentetik gen kümelerini tespit etmek amacıyla kapsamlı bir genom analizi antiSMASH sunucusunda (https://antismash.secondarymetabolites.org/#!/start) gerçekleştirilmiştir. *Streptomyces* sp. BSP1'in tüm genom dizisi terpen, poliketid sentaz, ribozomal olmayan polipeptid sentetaz, lantipeptid, tiyopeptid ve siderofor kodlayan gen kümeleri içermektedir. Antimikrobiyal aktivite testi, *Streptomyces* sp. BSP1 tarafından sentezlenen gümüş nanopartiküllerinin *Bacillus cereus* EMC15 ve *Candida albicans* ATCC 10231'in gelişimini inhibe ettiğini göstermiştir. Sonuç olarak, *Streptomyces* sp. BSP1 tarafından sentezlenen gümüş nanopartiküllerinin farmasötik endüstride kullanım alanı olabileceği düşünülmektedir.

**Anahtar kelimeler:** Antimikrobiyal aktivite, Genom analizi, Gümüş nanopartikülleri, Nanobiyoteknoloji, *Streptomyces*.

# ABSTRACT

## BIOACTIVITY CHARACTERISTICS OF SILVER NANOPARTICLES SYNTHESIZED BY *STREPTOMYCES* SP. BSP1

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The members of the phylum *Actinobacteria* are considered one of the most prolific microbial groups because of their high potential for synthesis of various bioactive metabolites. Especially endophytic actinobacteria are characterized by high secretion of proteins, enzymes and secondary metabolites that promote nanoparticle synthesis by reducing activity. In this thesis study, *Streptomyces sp*. BSP1 strain isolated from a legume nodule in a previous study was used to synthesize silver nanoparticles. The synthesized silver nanoparticles were confirmed by UV-vis spectroscopy, FTIR, XRD, SEM and EDX analyses. The UV-vis spectrum analysis exhibited maximum absorbance peak at 439 nm. The SEM analysis showed that the nanoparticles have regular spherical shapes and their average size ranges between 7.7 nm and 15.3 nm. The XRD analysis revealed that the synthesized silver nanoparticles have crystalline structure. In addition, the whole genome sequence of *Streptomyces sp*. BSP1 was downloaded from the NCBI GenBank to conduct a comprehensive genome analysis. The genome sequence of *Streptomyces sp*. BSP1 was annotated on the RAST server (https://rast.nmpdr.org/rast.cgi) and found to be about 7.4 Mb with 73.1% GC content. The number of protein coding sequences and RNAs were determined as 6868 and 76, respectively. From a phylogenetical point of view, the comparative genome analysis of *Streptomyces* sp. BSP1 performed on the TYGS (https://tygs.dsmz.de/) revealed that *Streptomyces* sp. BSP1 was closely related to *Streptomyces albidoflavus* NRRL B-1271 by sharing the highest digital DNA- DNA hybridization value of 65.2%, which is lower than the threshold value of 70% to delineate genomic species. Moreover, a comprehensive genome analysis was performed on the antiSMASH server (https://antismash.secondarymetabolites.org/#!/start) to reveal the biosynthetic gene clusters for secondary metabolites. The genome of *Streptomyces sp*. BSP1 has gene clusters encoding for terpenes, polyketide synthases, non-ribosomal peptide synthetases, lanthipeptides, thiopeptides and siderophores. The antimicrobial activity analysis showed that the silver nanoparticles synthesized by *Streptomyces* sp. BSP1 inhibited the growth of *Bacillus cereus* EMC15 and *Candida albicans* ATCC 10231. Consequently, it is considered that the silver nanoparticles synthesized by *Streptomyces sp*. BSP1 may have applications in pharmaceutical industry.

**Keywords:** Antimicrobial activity, Genome analysis, silver nanoparticles, Nanobiotechnology, *Streptomyces*.

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Heba ZAHRAN

## CONTENTS

# SYMBOLS AND ABBREVIATIONS

| | |
|---|---|
| AgNPs: | Silver nanoparticles |
| FRAP: | Ferric Reducing Antioxidant Power |
| DPPH: | 2,2-Diphenyl-1-picrylhydrazyl |
| ROS: | Reactive Oxygen Species |
| G+C: | Guanine and Cytosine |
| TYG: | Tryptone-Yeast Extract Agar |
| GYM: | Glucose-Yeast extract-Malt extract-agar |
| TSB: | Tryptone Soy Broth |
| RAST: | Rapid Annotations Using Subsystems Technology |
| TYGS: | Type (Strain) Genome Server |
| GBDP: | Genome Blast Distance Phylogeny |
| AntiSMASH: | Antibiotic and Secondary Metabolite Analysis Shell |
| NCBI: | National Center for Biotechnology Information |
| UV-Vis: | Ultraviolet-Visible spectroscopy |
| SEM: | Scanning Electron Microscopy |
| EDX: | Energy Dispersive X-Ray Spectrometry |
| XRD: | X-Ray Diffraction |
| FTIR: | Fourier Transform Infra-Red |
| dDDH: | digital DNA-DNA hybridization |
| SPRs: | Surface Plasmon Resonances |

# FIGURES LEGENDS

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# 1. INTRODUCTION

## 1.1. Nanotechnology

The term "nanotechnology" refers to a wide range of study in which the typical dimensions are less than 100 nanometers. From lithography (with line widths less than one nanometer) to nanomachines and nanorobots, this definition includes a wide range of scientific subjects. Nanotechnology includes the construction of nanostructures and the production of nanometer-scale things. As a result, DNA computation (as nanoscale particle manipulation), various types of microscopy, molecule manipulation, nanometer resolution diagnostic and analytical equipment, and nanomachines and nanorobots can all be classified as nanotechnology (Bogunia-Kubik and Sugisaka, 2002). In other words, the technologies implemented at the nano scale and have real-world applications are referred to as nanotechnology. It is described as the manipulation or reorganization of matter at the atomic and molecular level, ranging in particle size from one to one hundred nanometers. Matter has different properties on a nanoscale than on a bigger scale. When reducing a material from a big size, the material properties initially remain constant, after those tiny changes occur. Eventually, if the particle size is less than one hundred nm, the properties can change dramatically. The different physico-chemical properties of nanomaterials can be exploited for both commercial applications and novel services for the benefit of society. Nanotechnology includes the manufacture, application and integration of biological, physical and chemical systems on the scale of single molecules or atoms down to submicron dimensions and the incorporation of the resulting nanostructures into bigger systems. It has influenced our culture and economy in the 21st century in much the same way as semiconductor technology, information technology and cell and molecular biology did in the 20th century. Intelligent substances, electronics, nano-manufacturing, drug delivery, energy and water, biotechnology, information technology, and national security are just a few of the current environmental, medical, and industrial problems that have been solved by integrating nanotechnology into larger systems. Nanotechnology is a modern industrial revolution that will have a major impact on our economy and society. Nanotechnology is a megatrend that will bring disruptive innovation (Bhushan, 2017).

## 1.2. Nanobiotechnology

Nanotechnology research consider one of the most rapidly growing means of study, having administrations in science and technology for the synthesis of new nanoscale substances (Albrecht et al., 2006). The prefix nano comes from the Greek expression nanos, that means "dwarf," and relevant objects which are one billionth (10−9 m) in dimension. Professor Norio Taniguchi from Tokyo university of science created the expression nanotechnology in 1974 for explaining the nanometer-level accuracy of industrialized biomaterials (Taniguchi, 1974). Professor Richard P. Feynman, a physicist, presented the core notion of nanotechnology in his lesson “There’s plenty of room at the bottom” (Feynman, 1959). Nanobiotechnology has developed like a combination of nanotechnology and biotechnology for producing biosynthetic and environmentally co-friendly nanomaterial synthesis technic (Rai et al., 2009).

Nanomaterials typically range in size from 0.1-100 nm in each spatial dimension and are usually formed by one of two techniques: top-down or bottom-up (Schirmer, 1999). In the top-down mechanism, the bulk materials are progressively broken down into nanoscale particles, while in the bottom-up mechanism, atoms and molecules are assembled into nanometer-scale molecular structures. The bottom-up strategy was often utilized for the chemical and biological production of nanomaterials (Manivasagan et al., 2016).

Since the advent of nanotechnology, including molecular diagnostics, scientists have developed techniques to fabricate nano-scale particles and explored their back demand for the variety of administrations (Cao et al., 2002), catalysis (Pearson et al., 2010), electronics (Schmid and Corain, 2003), drug handing over (Kang et al., 2010), sensing (Nassif et al., 2004) and surface-enhanced Raman scattering (Plowman et al., 2010). The physical and chemical techniques for the critical synthesis of these technologically significant substances with accuracy in terms of form, dimension, and characteristics proliferated in these early stages of the field. However, because of the employment of toxic chemicals, harsh reaction conditions, and the emission of harmful byproducts into our environment, the matter of vital concern in relation to make use of these substances, for example, in the therapeutic administrations, has been rising up. As a result, developing a safe, clear, biocompatible, and environmentally co-friendly "green" way to production these technologically important substances are critical (Bansal et al., 2008).

Nanotechnology has made significant contributions to biomedical science, particularly in the bacterial therapy (Ahmad et al., 2014; 2015) and viral infections (Goel et al., 2021; Zeyaullah et al., 2021), neurological disorder (Ahmad et al., 2014), cancer therapy and drug delivery of medicine (Ahmad et al., 2021; Barani et al., 2021). Furthermore, due to the wide range of applications in biomaterials, medicine, food, pharmaceuticals, textiles and polymers, to name a few, the development and implementation of novel nano substances is increasing exponentially (Jeevanandam et al., 2018; Sardar et al., 2014).

### 1.3. Silver nanoparticles (AgNPs)

Silver nanoparticles (AgNPs) are silver particles with a diameter of less than 100 nanometers that are increasingly being exploited in medicinal applications due to their individual chemical, physical, and biological properties (Mude et al., 2009). However, silver nanoparticles could damage and finally kill bacterium cells by a variety of methods, including interactions with deoxyribonucleic acids (DNAs), enzyme deactivation, cell membranes and modifies the protein expression (Lok et al., 2006). These nanoparticles have antibacterial properties toward a variety of animal and human infections (Taglietti et al., 2012). Plenty of work has concentrated on the impact of silver nanoparticles on prokaryotic microbes, particularly pathogenic bacteria, while eukaryotic microbes have received less attention. However, Juganson et al. (2017) discovered that AgNPs are lethal to the protozoan *Tetrahymena thermophila.* Antibiotics combined with biogenic AgNPs have also been shown to boost antibacterial effect against *Pseudomonas aeruginosa, Escherichia coli*, and *Staphylococcus aureus* (Patra and Baek, 2017). AgNPs own a reduced hazard of generating antimicrobial resistance than antibiotics because of their non-specific antibacterial action (Smekalova et al., 2016). As a result, combining standard antibiotics with AgNPs is one of the approaches suggested to combat antimicrobial resistance. Even at very low concentrations of antibiotics (110 mg/l), the study has shown that such mixtures could suppress the development of harmful germs, comprising resistant strains (Panáček et al., 2009; Kvítek et al., 2008).

Many investigations have also shown that Gram-negative bacteria like *P. aeruginosa* (Ramalingam et al., 2014), *Stenotrophomonas maltophilia* (Oves et al., 2013), *E. coli* (Shahverdi et al., 2007), and *Enterococcus sp.* (Rajeshkumar et al., 2016) are capable of producing silver nanoparticles and developing anticancer characteristics. Scientists have even considered the biogenic production of silver nanoparticles, as well as their anticancer and antibacterial activities after being manufactured out of species like *Streptomyces xinghaiensis* OF1 (Wypij et al., 2018) and *Streptomyces naganishii* MA7 (Shanmugasundaram et al., 2013).

Nanoparticles are increasingly being used as an alternative to antibiotics to attack bacteria. Most techniques of antibiotic resistance are unrelated to nanosubstances since their effect occurs through straight interaction with the cell wall of the bacteria without any need for cell penetration; this suggests that nanoparticles promote bacterial resistance less than antibiotics (Wang et al., 2017). Because of their wide range of biological uses, such as drug delivery agents, antimicrobial resistance, tumor diagnostics and tumor therapy, silver nanoparticles have attracted a lot of attention across the world (Goel et al., 2021).

At nanoscales, silver nanoparticles (AgNPs) have various advantages: Photoelectrochemical activity, excellent conductivity, and enhanced stability are all advantages of having a high surface plasmon resonance (SPR). Because of its safety, eco-friendliness, and cost-effectiveness, the study concentrates on greenly manufactured nanomaterials, primarily utilizing fungi, bacteria and actinobacteria as nanofactories (Ahmad et al., 2015; Mishra et al., 2016).

### 1.3.1. Silver Nanoparticles and Their Activity Properties

#### 1.3.1.1. AgNPs Synthesis

Various approaches (Kowshik et al., 2002) can be used to synthesize Ag nanoparticles, which are recognized for their high antibacterial activity**.** These methods might be classified as physical, chemical, or biological in general. Some of these approaches are straightforward and allow for effective control of nanoparticle size by adjusting the reaction process; however, there are still issues with product stabilization and achieving unimodal distribution in the nano-region (Vilela et al., 2015).

#### 1.3.1.1.1. Physical Methods

Evaporation-condensation is one of the physical methods for making AgNPs, although it has several drawbacks, such as high energy consumption and a long time to reach thermal stability (Kowshik et al., 2002; Baláž et al., 2020). Various physical procedures were developed as a result of these factors. Pluym et al. (1993) employed spray pyrolysis, which is a common method of pyrolysis (Zhang et al., 2016). Lee and his colleagues (2004) created nanoparticles that were thermally decomposed (Gurav et al., 1994), or Baláž (2018) employed ball milling to great effect. This approach, also known as mechanochemical synthesis, has recently gained many concentrations in the community of science (Lee and Kang, 2004; Do and Friščić, 2017). Physical procedures are advantageous because of their quickness and lack of hazardous chemicals, but they also have drawbacks, as previously stated (Baláž et al., 2020).

#### 1.3.1.1.2. Chemical Methods

Chemical techniques offer a quick and easy way to prepare AgNPs in solution. The reduction of organic or inorganic reducing agents such as sodium ascorbate, hydrogen, N,N-dimethylformamide, or sodium borohydride considered the mostly wide mechanism for the formation of nanoparticles. AgNPs synthesis is based on the reduction of the $Ag^+$ ion to the metallic form $Ag^0$, which is then agglomerated into oligomeric clusters, resulting in metallic colloidal AgNPs (Baláž et al., 2020). To prevent nanoparticle aggregation, stabilizing chemicals such as poly(vinyl alcohol), poly(vinylpyrrolidine), or polyethylene glycol must be used (Xu et al., 2020).

#### 1.3.1.1.3. Biological Method

Biological nanoparticle production is winning ground because of its simplicity and low cost as well as its environmental benefits. A natural object such as plants, microorganisms or biological molecules such as amino acids or polysaccharides reduces metal salts (Baláž et al., 2017). Biological approaches can be classified as *in vivo* or *in vitro* depending on the methodology used. *In vivo* methods utilize the whole cell for the production of AgNPs, hence nanoparticles are produced intracellularly or extracellularly, however, *in vitro* methods reduce $Ag^+$ ions outside of a living organism. Plant extracts containing antioxidants and reducing chemicals such as polyphenols are the most popular, flavonoids, terpenes, aldehydes, carbohydrates, etc. (Gurunathan et al., 2009). In addition to plant extracts, edible mushroom extracts

(Mittal et al., 2013), microorganisms extract, cellulose, starch, pectin, dextrin can be put to use (Bedlovičová et al., 2020).

The biological synthesis of AgNPs inside a living creature is referred to as *in vivo*. Gardea-Torresdey et al. (2002) presented the first paper, which described the synthesis of AgNPs by an alfalfa. They discovered that the alfalfa root can absorb silver from agar media and convert it to AgNPs (Konował et al., 2017). Nanoparticles can also be produced by microorganisms. Because some microorganisms are metal-resistant, they can survive and proliferate throughout the formation of NPs, therefore using bacteria to make AgNPs is not a problem (Pivec et al., 2017). Because of their propensity to bioaccumulate metals, fungi are also a valuable source of silver nanoparticles (Singh et al., 2018).

#### 1.3.1.2. Silver Nanoparticles as Antimicrobial Agent

Because of their relatively huge surface area allowing superior connection with bacteria, silver nanomaterials have better antimicrobial activity compared to other salts. The nanosubstances connect to the membrane of the cell then also pass through inside the bacteria. Proteins having sulfur were located in the membrane of the bacteria, with which silver nanoparticles and phosphorus-containing substances such as DNA interact. When AgNPs enter the cell of the bacteria, they create an area of faint molecular weight inside the core of the microorganism where the organism combines and protects DNA from Ag ions. The nanosubstances preferentially attack the respiratory chain, which causes division of the cell then eventually killing the cell. In the cells of the organism, nanosubstances produce Ag ions that improve their bactericidal action (Morones et al., 2005).

The surface plasmon resonance, which switches to an extended wavelength with increasing particle dimension, is important for determining the spectra of optical absorption of silver nanosubstance. Because of their small size, the nanoparticles have a vast superficial area for interacting with the cell of the organism, resulting in an upwards of proportion of interconnection than larger substances (Pal et al., 2007). Nanosubstances fewer than ten nm interplay with the bacteria and cause electrical impacts that increase the action of the nanosubstances. The fact that the bacterial death impact of Ag nanomaterials is dimension interdependent is thus confirmed (Raimondi et al., 2005). The antimicrobial action of nanomaterials is also related to their forms, as evidenced by studies of the growth of the bacteria which inhibited through variably shaped nanomaterials (Morones et al., 2005). With a silver content of 1 g, truncated triangular nanoparticles show bacterial suppression (Pal et al., 2007). As a result, different shapes of silver nanoparticles have diverse impacts on bacterial cells (Rai et al., 2009).

#### 1.3.1.3. Anti-inflammatory Effect of Silver Nanoparticles

The antibacterial property of silver nanoparticles is most commonly used in the medical field, but the anti-inflammatory property is also widely used. Preliminary research suggests that wound healing is accelerated in the presence of nanoparticles (Kirsner, 2002). When silver nanoparticles were injected into a mouse model of burn injuries, levels of pro-inflammatory cytokines were reduced (Tian et al., 2007). Interferon gamma and tumor necrosis factor alpha were also inhibited by silver nanoparticles. Although these studies show that silver nanoparticles play a role in anti-inflammatory effects, the exact mechanism of action is still unknown. Nanosilver, on the other hand, has anti-inflammatory properties, making it a great option for use as an anti-inflammatory in a variety of therapies (Prabhu and Poulose, 2012).

#### 1.3.1.4. Antioxidant Effects of Silver Nanoparticles

Antioxidant properties of numerous biological samples, chemicals, and isolated molecules are well understood. In recent decade, a considerable number of studies dealing with the antioxidant abilities of silver nanoparticles have been published, in addition to its application in many fields.

The antioxidant activities of green-synthesized AgNPs are measured by using the DPPH free radical scavenging and FRAP assays. Because DPPH is a stable molecule that may be reduced by receiving electrons or hydrogen, it is commonly employed to measure antioxidant activity. The inverse relationship between $IC_{50}$ and DPPH scavenging activity has been found. Metal-derived free radicals produce oxidative stress; for example, reactive oxygen species (ROS) in bacteria destroy cell walls, DNA, and mitochondria, eventually leading to cell death (Konappa et al., 2021) (Figure 1.1).

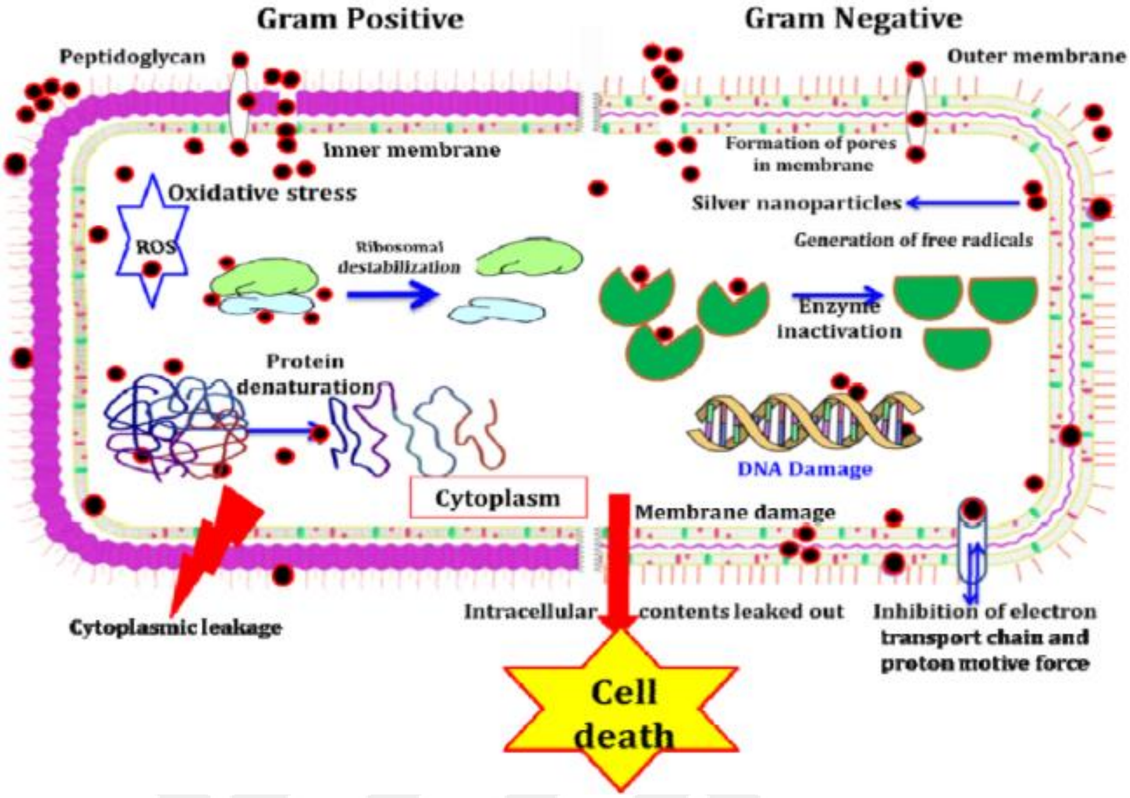

Figure 1.1. Proposed mechanisms for antimicrobial activity of silver nanoparticles (Konappa et al. 2021)

### 1.4. Green Synthesis of Metallic Nanoparticles

Several physicochemical methods are used for the synthesis and manipulation of metal nanoparticles. Evaporative condensation and laser ablation are two popular physical methods to produce tiny, stable nanoparticles in high concentration with uniform distribution and minimal solvent contamination. Chemical reduction with inorganic and organic reducing agents is a typical approach to prepare AgNPs. These agents convert $Ag^+$ to $Ag^0$, which is then protected and stabilized by polymeric substances. These preservatives allow the development of nanoparticles while preventing agglomeration, sedimentation and the consequent loss of surface qualities. Microemulsion processing, UV-initiated photoreduction, photochemical reduction, electrochemical synthesis, and microwave-assisted synthesis are some of the other chemical routes. However, all of these approaches suffer from a number of disadvantages. These technologies are extremely expensive and polluting as they require a lot of energy and use toxic, dangerous, harsh and flammable chemicals and heavy metals. Separation and removal of large volumes of reducing agents, surfactants and organic solvents from the final mixture is also required. Low reproducibility and long-term viability of these methods, as well as difficulties in scaling leading to lower production, and issues with nanoparticle growth, size, shape, and structural deformation have led to an increasing need to discover appropriate alternatives for effective synthesis of nanoparticles.

To overcome these shortcomings, green nanoparticle synthesis employs processes that are cost-effective, dependable, repeatable, sustainable, and environmentally benign, while avoiding or minimizing undesirable, harmful by-products. In the biogenic production of nanoparticles, extracts from biological sources act as protective substances. Irradiation of precursor silver salt solutions with a laser, mercury lamp, or visible light resulted in AgNPs with a well-defined shape and uniform distribution. Biological methods using unicellular and multicellular organisms such as bacteria, fungi, yeast, viruses, and plants (or plant-derived extracts) have been explored over the past two decades as potential alternatives to chemical and physical methods to produce nanoparticles (Paul and Roychoudhury, 2021).

### 1.4.1. Biological Components for Green Synthesis of Metallic Nanoparticles

Many physical and chemical synthesis processes require high levels of radiation, highly toxic reducing agents and stabilizers, all of which can be harmful to humans and marine life. Green synthesis of metallic nanoparticles, on the other hand, is a green one-pot or one-step bioreduction approach that requires relatively little energy to start with it. This type of reduction is also inexpensive (Dahoumane et al., 2016).

#### 1.4.1.1. Bacteria

Commercial biotechnology fields such as biological remediation, genetic engineering, and bioleaching have benefitted from bacterial species. Bacteria have the ability to degrade metal ions and are important candidates for the production of nanoparticles. A number of bacterial species are used in the synthesis of metallic and other novel nanoparticles. Metal or metal oxide nanoparticles have been synthesized on a large scale using prokaryotic bacteria and actinomycetes. Since bacteria are relatively easy to manipulate, bacterial nanoparticle production was taken up. Several bacteria have been known to synthesize biologically reduced silver nanoparticles with various size and shape morphologies: *Arthrobacter gangotriensis*, *Aeromonas sp.* SH10, *Bacillus amyloliquefaciens*, *Bacillus cereus*, *Bacillus cecembensis*, *Bacillus indicus*, *Corynebacterium sp.* SH09, *Escherichia coli*, *Geobacter sp.*, *Lactobacillus casei*, *Phaeocystis antarctica*, *Pseudomonas proteolytica*, *Enterobacter cloacae* and *Shewanella oneidensis*. Similarly, various bacterial species, for example, *Bacillus* *megaterium* D01, *Desulfovibrio desulfuricans*, *E. coli* DH5a, *Bacillus subtilis* 168, *Shewanella alga*, *Rhodopseudomonas capsulata* and *Plectonema boryanum* UTEX 485, are used to synthesize gold nanoparticles and have been widely utilized (Thakkar et al., 2010).

#### 1.4.1.2. Fungi

The biological synthesis of metal or metal oxide nanoparticles by fungi is another highly efficient approach to produce monodisperse nanoparticles with uniform morphologies. Since they contain a number of intracellular enzymes, they are better biological agents for the production of metal or metal oxide nanoparticles. Some fungi can produce more nanoparticles compared to bacteria. In addition, fungi have numerous advantages over other organisms because of the presence of enzymes, proteins, and reducing components on their cell surfaces. The enzymatic reduction employing a reductase in the cell wall or inside the fungal cell is the most proper mechanism for the formation of metallic nanostructures. A variety of fungal microorganisms are employed to produce metal or metal oxide nanoparticles such as gold, silver, titanium dioxide and zinc oxide (Narayanan and Sakthivel, 2011).

#### 1.4.1.3. Yeast

Yeasts are single-celled bacteria that live inside eukaryotic cells. There have been 1500 yeast species identified so far. Several researchers have reported the successful fabrication of nanoparticles/nanomaterials using yeasts. A silver-tolerant yeast strain and *Saccharomyces cerevisiae* culture broth were used for the biosynthesis of silver and gold nanoparticles. Various yeast species are employed to manufacture a wide range of metallic nanoparticles (Yurkov et al., 2011).

#### 1.4.1.4. Plants

Plants have the ability to accumulate heavy metals in some areas of their bodies. As a consequence, biosynthetic techniques employing plant extracts have attracted high attention as a simple, efficient, inexpensive, and practical way to fabricate nanoparticles, and as an excellent alternative to traditional manufacturing methods. In a "one-pot" proliferation of procedure, a range of plants can be employed to decrease and stabilize metallic nanoparticles. To further explore the many applications of metal or metal oxide nanoparticles made from plant leaf extracts, many researchers have used an environmentally friendly manufacturing approach. Plants contain biological molecules such as carbohydrates, proteins, and coenzymes which have high potential for reducing metal salts to nanoparticles. Gold and silver metal nanoparticles, like other biosynthetic methods, were first explored in plant extract-assisted synthesis. Silver nanoparticles and gold nanoparticles were synthesized using a variety of plants including *Aloe barbadensis* Mill., *Avena sativa* L., *Azadirachta indica* A. Juss., *Citrus limon* (L.) Osbeck, *Coriandrum sativum* L., *Medicago sativa* L., *Ocimum tenuiflorum* L. Metallic nanoparticles can be generated through the reduction of metal salt ions, which are absorbed as soluble salts in living plants (*in vivo*). The *in vivo* formation of nanoparticles such as zinc, cobalt, copper and nickel has also been reported in mustard, alfalfa and sunflower (Marchiol, 2012).

### 1.5. *Actinobacteria*

Gram-positive bacteria with a GC-rich linear genome and a powerful biosynthetic ability to create secondary metabolites with a wide structural variety and commercial value are known as *Actinobacteria*. The majority of this phylum's species have a complicated mycelial lifecycle, forming aerial and substrate mycelium with distinct coloration and sporulation (Figure 1.2).

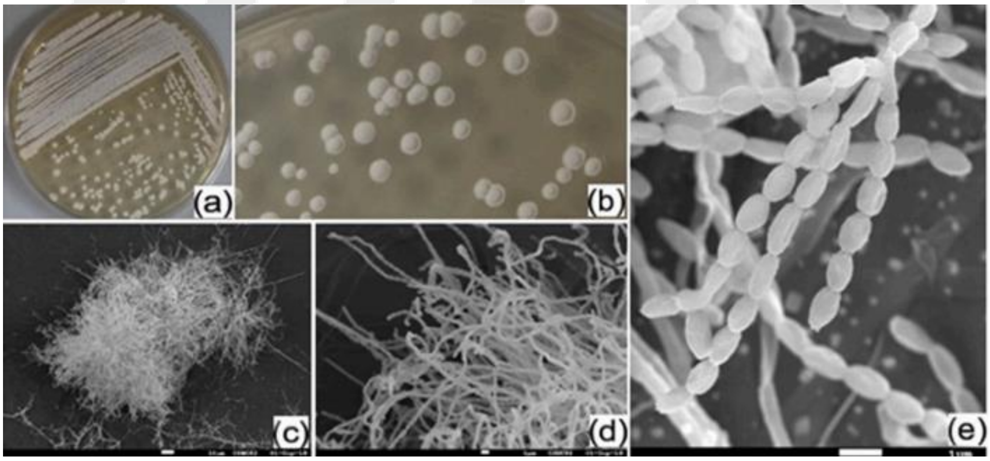

Figure 1.2. Actinobacterial lifestyle and morphological appearance. a, b: colony morphologies, c, d: SEM images showing filamentous growth, e: SEM image of spores in spore chains (Barka et al. 2016).

*Actinobacteria* can be found in a wide range of soils, freshwater and marine environments, plants, and animals. The *Actinobacteria* play critical functions in nutrient recycling and defense in their environment or linked host. They live as endophytes in plant tissues, where they help in nutrition absorption and growth. *Actinobacteria* colonize the outside or inside of insects, protecting the host and its nutritional supplies against pathogens. Furthermore, they can persist in difficult environments (e.g., the deep ocean) and promote nutrient cycling. *Actinobacteria* have evolved varied metabolic potentials as a result of their adaptation to such diverse habitats and interactions, allowing them to create a variety of secondary metabolites (Jose et al., 2021). As a result, only a few model organisms have been used to study actinobacterial biology, which are either medically significant pathogens like *Mycobacterium* or biotechnologically important producers like *Streptomyces*. Following the discovery of streptomycin, *Actinobacteria* have become a prolific producer of secondary metabolites in the search for novel medications. This group of bacteria accounts for half of all secondary metabolites generated from microorganisms, with over 22,000 of them described (Rao and Dhulappa, 2022).

### 1.5.1. Biotechnological Applications of *Actinobacteria*

*Actinobacteria* are known to produce primary and secondary metabolites with significant applications in a range of areas. They are also a potential source of a wide range of essential enzymes which are produced on a large scale. Moreover, *Actinobacteria* can create enzyme inhibitors for cancer treatment as well as immunomodulators that enhance the immune response. They can remove a wide range of complex polymers such as hydrocarbons, insecticides, aliphatic and aromatic chemicals, among others. Several members of the phylum *Actinobacteria* have the potential to be used in the biological conversion of underutilized agricultural and urban waste into valuable chemical compounds. They perform microbial transformations of organic molecules, which is a valuable sector. This group of bacteria plays an important role in plant biotechnology as strains with antimicrobial activity against plant diseases are useful for biocontrol (Ranjani et al., 2016). We offer a brief explanation of some of the most important *Actinobacteria* applications (Figure 1.3).

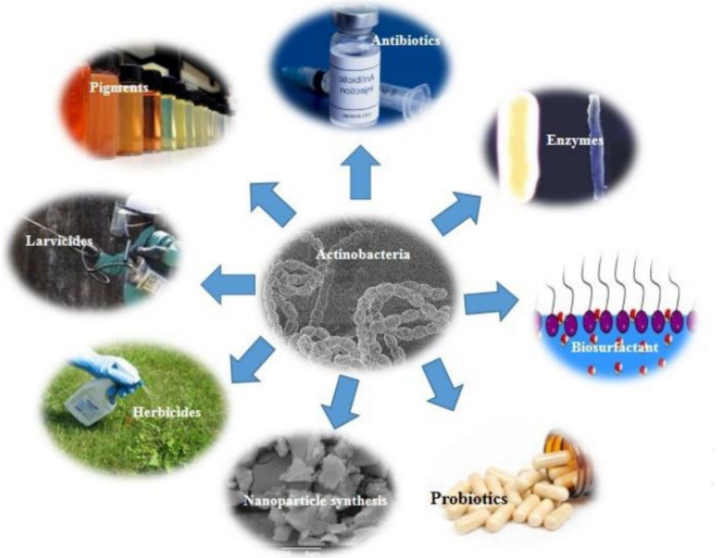

Figure 1.3. A brief explanation of some of the most important applications for actinobacteria (Gopinath et al. 2015).

### 1.5.1.1. Antimicrobials

*Actinobacteria* play a crucial role in the manufacture of a variety of medicines that are vital to our health and nutrition. Natural products with novel structures have been observed to exhibit biologically useful features. Natural sources will always be the most promising and adaptable entities of novel antibiotics though there have been important advances in the disciplines of chemical synthesis and engineered biosynthesis of antibacterial chemicals. Although thousands of antibiotics have been found to date, their toxic nature has limited their use. To solve this problem, research groups are searching for novel antimicrobial molecules which are both more effective and have no dangerous side effects. Antibiotics are mainly produced by microbes, especially *Actinobacteria* species. *Actinobacteria*, mainly the members of *Streptomyces* and *Micromonospora* species, are known to be the source of about 80% of the world's antibiotics. *Streptomyces* are the main antibiotic-producing organisms used by the pharmaceutical industry as they produce over 7600 chemicals, many of which are secondary metabolites that are potent antibiotics (Jensen et al., 1991).

#### 1.5.1.2. Enzymes

*Actinobacteria*, both marine and terrestrial, produce a wide range of physiologically active enzymes. They produce amylases outside the cells, which support extracellular digestion and have biotechnological applications such as in the food industry, fermentation, textile and paper industries. Cellulases are a group of hydrolytic enzymes that hydrolyze the glucosidic bonds of cellulose and related oligosaccharide derivatives, and they are another key component of *Actinobacteria*. Lipase is produced by a variety of *Actinobacteria*, other bacteria, and fungi and is employed in detergent, food, oleochemical, and diagnostic applications, as well as pharmaceutical industries. *Actinobacteria* have been obtained from a wide range of natural habitats including plant tissues and rhizospheric soil. Biological functions of this group of bacteria are largely determined by the sources from which they are isolated. Multiple proteases are known to be secreted by *Actinobacteri*a, particularly streptomycetes, in the culture medium (Schmid and Verger, 1998).

#### 1.5.1.3. Bioherbicides

The use of actinobacterial secondary metabolites as herbicidal agents against undesirable herbs and weeds is another potential application. Anisomycin is produced by a *Streptomyces* strain, and is effective as a growth inhibitor for annual grassy weeds such as barnyard grass and crabgrass and broadleaf weeds. Anisomycin can prevent plants from synthesizing chlorophyll. Similarly, bialaphos, a *Streptomyces viridochromogenes* metabolite that inhibits glutamine synthesis, is commonly employed to suppress annual and perennial grassland weeds as well as broad-leaved weeds (Xiliang, 1994).

#### 1.5.1.4. Probiotics

Probiotics are live microbial additives that benefit the host through a variety of mechanisms, including modifying the microbial community associated with or surrounding the host, providing improved feed efficiency or nutritional value, enhancing the host's disease response, and enhancing the quality of the host's environment. Several actinobacterial strains have also been reported as beneficial sources as probiotics (You et al., 2007).

#### 1.5.1.5. Biosurfactants

Biosurfactants are microbially generated molecules with surface active hydrophilic and hydrophobic moieties. In contrast to chemically produced surfactants, biosurfactants do not require mineral oil as a raw material, are easily biodegradable and can be produced at low temperatures. Biosurfactants are used in a variety of industries including nutrition, cosmetics, textile, paint, pharmaceutical, mining and oil extraction. Cubist Pharmaceuticals has marketed the lipopeptide antibiotic daptomycin, an actinobacterial biosurfactant that has previously been commercialized and is used to treat diseases caused by Gram-positive infections (Henkel et al., 2012).

#### 1.5.1.6. Vitamins

Bacteria or *Actinobacteria* may create vitamin B12 in its natural state. The extraction of vitamin B12 from the members of *Actinobacteria* fermentations sparked a lot of interest in microbial fermentations as a source of vitamins. When cobalt is added to the medium, the fermentations that produce the antibiotics streptomycin, aureomycin, grisein, and neomycin also produce some vitamin B12 without affecting antibiotic yield. Several other investigations have suggested that non-antibiotic producing *Actinobacteria* produce more of this vitamin than antibiotic-generating *Actinobacteria* (Rickes et al., 1948).

#### 1.5.1.7. Pigments

Microbe-oriented pigments are of great importance because synthetic dyes have some limitations, such as the use of hazardous chemicals in their manufacture, occupational safety issues, and the formation of hazardous waste. *Actinobacteria*, in particular, are characterized by the production of diverse colors on natural or synthetic surfaces, which is considered an essential cultural trait in defining the organisms (Gopinath et al., 2015).

#### 1.5.1.8. Nanoparticle synthesis

Because nanoparticles span the gap between bulk materials and atomic or molecular structures, they are of great scientific interest. Chemical processes are often low-cost for large volumes, but they have downsides such as contamination resulting from precursor chemicals, the use of hazardous solvents, and the formation of toxic byproducts. As a result, there is a growing tendency to develop high-yield, low-cost, nontoxic, and ecologically friendly metallic nanoparticle production processes. As a result, the biological approach to nanoparticle manufacturing becomes critical. *Actinobacteria* are capable of producing nanoparticles with a variety of biological activities, including antimicrobial, anticancer, antibiofouling, antiparasitic, and antioxidant properties. The members of *Streptomyces* and *Arthrobacter* have been investigated as potential nano-factories for the development of clean and non-toxic silver and gold nanoparticle production processes. Gopinath et al (Gopinath et al., 2015), reported the manufacture of silver nanoparticles from a *Streptomyces sp*. Ranjani et al. (2016) studied the biodiversity of silver nanoparticle synthesizing-*Actinobacteria* from the marine environment, finding that 25 isolates synthesized silver nanoparticles, including *Actinopolyspora* sp., *Actinomadura* sp., *Kitasatosporia* sp., *Nocardiopsis* sp., *Streptomyces* sp., *Thermoactinomyces* sp., *Kibdelosporangium* sp., *Saccharopolyspora* sp., and *Thermomonospora* sp. (Gopinath et al., 2015).

#### 1.5.1.8.1. The Role of *Actinobacteria* in Synthesis of Silver Nanoparticles

Nanotechnology is one of the most important technologies of the 21st century, and it is a hot-bed of study for particle sizes ranging from one to one hundred nanometers. Silver has been valued for its antibacterial and wound-healing qualities since antiquity. By 2025, global silver nanoparticle production is expected to be in the range of 360–450 tons per year, with an increase to 800 tons per year. Researchers have begun to investigate *Actinobacteria* in the field of nanotechnology because of their ability to synthesize a wide spectrum of biologically active chemicals (Huang et al., 2020).

*Actinobacteria* are common bacteria that create secondary metabolites. The antibiotic revolution began with the discovery of streptomycin from *Actinobacteria*, and actinobacterial members, particularly those of the genus *Streptomyces*, are now recognized as key producers of a large selection of bioactive metabolites. *Streptomyces* is the most common and prominent actinobacterial genus found in many ecological environments. Many actinobacterial members have been known to synthesize silver nanoparticles, but the bulk of data points to *Streptomyces* as the prominent category in the multiple ecological niches that might synthesize silver nanoparticles (Ranjani et al., 2016).

The entire procedures for production of silver nanoparticles using *Actinobacteria* are summarized in Figure 1.4. The actinobacterial strains are cultured in a suitable liquid medium under optimal growth conditions in general. Following the growth, the medium-free biomass or the culture-free extract are centrifuged and treated with $AgNO_3$. The color shift of the solution (from colorless to brown) is preliminary proof of the creation of silver nanoparticles (Anasane et al., 2016).

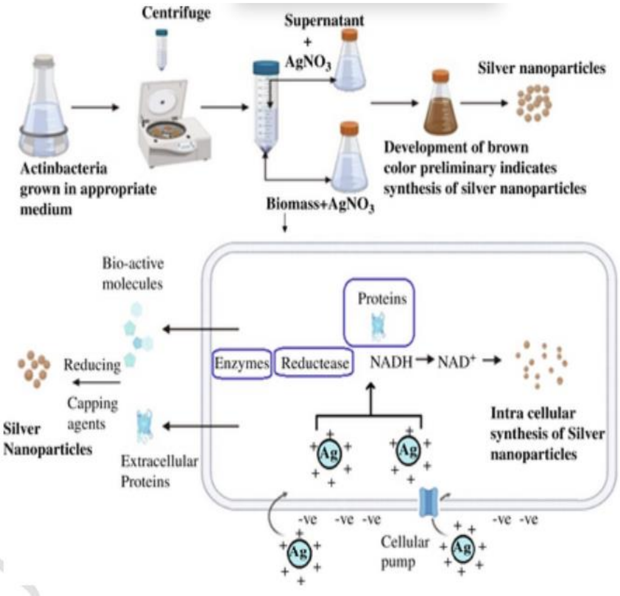

Figure 1.4. General steps proposed for synthesis of silver nanoparticles by *Actinobacteria* (Rao and Dhulappa 2022).

The intracellular or extracellular route of formation of silver nanoparticles is one possibility. Bacterial cells are treated with $AgNO_3$ and incubated at optimal growth conditions during intracellular synthesis. To avoid the involvement of medium components, bacterial cells are rinsed with sterilized distilled water prior to interaction. Due to the electrostatic interaction between $Ag^+$ ions and the negatively charged cell wall, $Ag^+$ ions are trapped at the surface, followed by reduction (by proteins, enzymes, etc.) and the formation of silver nanoparticles behind the cell wall. According to a study by Otari et al. (2015), it has also been suggested that proteins along with other bioorganic compounds could form a coating that prevents aggregation of silver nanoparticles.

Similarly, Dong et al. (2017) reported that a cell-free actinobacterial extract which contains proteins, enzymes and other bioactive compounds was treated with $AgNO_3$ and following the incubation the silver nanoparticles were formed. A reducing and stabilizing agent was found in the cell-free extract which contained proteins, enzymes, and other bioactive molecules (Dong et al. 2017).

#### 1.5.1.8.2. Factors Affecting Silver Nanoparticle Synthesis

There are reports on the microorganisms' responses to physicochemical factors (Figure 1.5). They appear to play a critical role in the synthesis of silver nanoparticles when they are mediated. Silver nanoparticles were created in just 90s when microwave irradiation was utilized instead of photo-irradiation, demonstrating that the synthesis parameters are equally significant for the rapid synthesis of silver nanoparticles. During the synthesis of silver nanoparticles, the reducing agent's stability is critical. The synthesis was seen even at high temperatures since the reducing agent was thermostable, and with temperature increases up to 100°C, stable and fast nanoparticle synthesis was observed (Kiran et al., 2014).

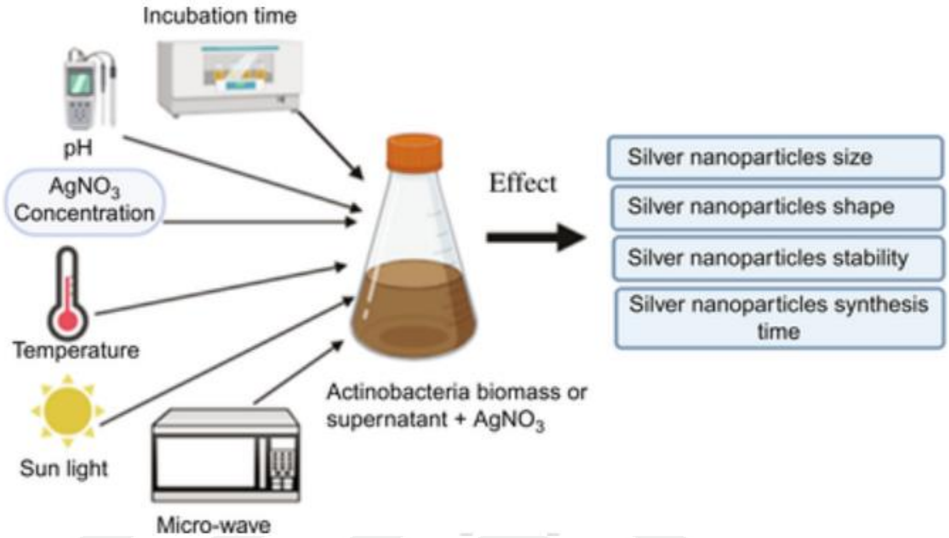

Figure 1.5. Factors affecting the synthesis of silver nanoparticles (Rao and Dhulappa, 2022).

Antimicrobial properties of silver have been known since ancient times when it was used to conserve water and wine as well as used to heal wounds. Compared to their bulk starting materials, silver nanoparticles have unique physical, chemical and biological properties. Silver nanoparticles made from actinobacterial strains have a wide range of uses in a variety of fields. *Streptomyces albidoflavus*-derived silver nanoparticles were found to exhibit antibacterial activity against both Gram-negative and Gram-positive strains of bacteria. According to the reports, when silver nanoparticles are mixed with antibiotics, their antibacterial activity is improved. The cytotoxicity activity of silver nanoparticles generated by *Streptomyces* sp. (09 PBT 005) against the A549 adenocarcinoma lung cancer cell line was 83.2 %. Exopolysaccharide from *Streptomyces violaceus* MM72 was used to make silver nanoparticles, which had antibacterial and antioxidant properties (Sivasankar et al., 2018).

# 2. MATERIALS AND METHODS

## 2.1. Synthesis of Silver Nanoparticles

A total of 10 strains were activated on Tryptone-Yeast Extract Agar (TYG) (Tryptone 3 g/l, yeast extract 5 g/l, glucose 5 g/l, 20 g/l, distilled water 1000 ml, pH 7.0) and Glucose – Yeast extract – Malt extract agar (GYM) (glucose 4 g/l, yeast extract 4 g/l, malt extract 10 g/l, CaCO3 2 g/l, agar 12 g/l, distilled water 1000 ml, pH 7.2) media and tested for their ability to synthesize silver nanoparticles. After activation on agar media, colonies were picked and transferred to a 30 ml Tryptone Soy Broth (TSB) (Oxoid) medium to prepare an inoculum. The broth cultures were incubated at 28°C for 5-7 days in a shaking incubator and then a volume of 5 ml for each strain was used to inoculate 100 ml of TSB medium. The inoculated flasks were incubated at 28°C for 5-7 days in a shaking incubator. After incubation, the culture broths were centrifuged at 10,000 rpm (4°C) and the supernatants collected for nanoparticle synthesis. To synthesize silver nanoparticles, 2 mM $AgNO_3$ solution (100 mL) was mixed with an equal volume of culture supernatants and stirred constantly. The supernatant – $AgNO_3$ solutions were then placed in a shaking incubator (150 rpm) and the colour change from yellow to brown-black was observed within a week. The strain *Streptomyces* sp. BSP1 isolated from a legume nodule (Ay 2020) was the most effective in terms of synthesis time and colour darkness. The silver nanoparticles were collected by centrifugation (13,000 rpm, 4°C) and washed twice with sterile distilled water. The silver nanoparticles were dried prior to analysis.

## 2.2. Genome Annotation of Strain *Streptomyces* sp. BSP1

The genome sequence data of strain *Streptomyces* sp. BSP1 was downloaded from the NCBI GenBank database (accession number JAMGZL000000000) and annotated on the Rapid Annotations Using Subsystems Technology (RAST) server (Aziz et al., 2008). For phylogenomic analysis, the genome sequence of strain *Streptomyces* sp. BSP1 was transferred to the Type (Strain) Genome Server (TYGS) at https://tygs.dsmz.de (Meier-Kolthoff and Göker, 2019). The secondary metabolite coding gene clusters of strain *Streptomyces* sp. BSP1 was determined by uploading the genome sequence to the Antibiotics and Secondary Metabolite Analysis Shell (antiSMASH) (https://antismash.secondarymetabolites.org/#!/start) (Blin et al., 2021) and analysed on ‘relaxed’ detection strictness.

## 2.3. Characterization of Silver Nanoparticles Synthesized by Strain *Streptomyces* sp. BSP1

The characterization studies on the morphology, size and composition of the silver nanoparticles synthesized under optimal conditions were performed using UV-Vis, FTIR, XRD, SEM and EDX.

### 2.3.1. UV-Vis Analysis

UV-Vis spectrophotometer was used for absorption or reflection spectroscopy in the ultraviolet-visible spectral range. The colour change of the silver nanoparticle synthesis was monitored visually and the absorbance was measured with a UV-Vis spectrometer (Thermo Scientific Evolution 201/220). Spectral studies were performed in the 200–800 nm range at 1 nm resolution using a quartz cuvette.

### 2.3.2. Scanning Electron Microscopy–Energy Dispersive X-Ray Spectrometry (SEM–EDX) Analysis of Silver Nanoparticles

The size, shape and elemental analysis of silver nanoparticles were confirmed by SEM (JEOL JSM-7001f). In addition, EDX analysis was applied to verify the elemental composition of the silver nanoparticles and identify signals for silver atoms. The ImageJ analysis software (Abràmoff et al., 2004) was used to determine nanoparticle size distribution.

### 2.3.3. Fourier Transform Infrared (FTIR) Analysis of Silver Nanoparticles

In order to characterize silver nanoparticles, all functional groups likely to be found on the surface of the nanoparticles synthesized by the strain *Streptomyces* sp. BSP1 were determined by FTIR spectroscopy (PerkinElmer Spectrum two). FTIR spectra were recorded in the range of 450-4000 cm$^{-1}$ at a resolution of 34 cm$^{-1}$.

### 2.3.4. X-Ray Diffraction Analysis of Silver Nanoparticles

X-ray diffraction analysis is mainly used to identify the crystalline structure and phase of materials such as minerals or inorganic compounds. The XRD analysis was performed after centrifugation and a powdered sample of synthesized AgNPs was deposited on a diffractometer glass substrate and treated at 40 kV voltage and 30 mA current using Cu-K radiation (wavelength of 1.5418) in the 2θ range from 5° operated to 100° at 2°/min scan speed.

### 2.4. Antimicrobial Activity of Silver Nanoparticles

Antimicrobial activities of the synthesized silver nanoparticles were tested against *Bacillus cereus* EMC15, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Enterobacter faecalis* ATCC 29212 and *Candida albicans* ATCC 10231 using drop diffusion method. The pathogen microorganisms were activated on nutrient agar medium at 37°C for 24 hours and then, each microorganism was suspended in sterile Ringer's solution (Oxoid) to obtain an optical density between 0.5-1.0 corresponding to $1.5\text{-}3.0 \times 10^8$ CFU/ml. An aliquot of 100 µl suspension was spread onto nutrient agar medium. After drying completely, 25 µl silver nanoparticle suspension (0.05 mg/ml) was dropped onto the agar. Sterile distilled water was used as negative control. Nystatin and rifampicin were used as positive control for yeast and bacteria, respectively. The plates were incubated at 37°C for 24 hours and inhibition zones were measured. The experiments were performed as duplicate.

### 2.5. Antioxidant Activity of Silver Nanoparticles

In order to analyse antioxidant capacity of the synthesized silver nanoparticles, DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity test was performed following the method described by Priya et al. (2010). The silver nanoparticles were suspended in sterile distilled water to prepare different concentrations (25 mg/ml, 12.5 mg/ml and 6.25 mg/ml). Two hundred microliter of each dilution was mixed with 100 µl 0.2 mM DPPH solution and incubated at 20°C for 40 min in dark. The absorbance was measured at 517 nm using a UV-visible spectrophotometer with methanol as a blank. Gallic acid was used as a positive control. The percentage scavenging of DPPH by the silver nanoparticles was calculated using the following formula:

$$\%DPPH\ radical\ scavenging\ activity = \frac{Ac - At}{Ac} x\ 100$$

$A_c$: Absorbance of the control

$A_t$: Absorbance of the test sample

# 3. RESULTS AND DISCUSSION

## 3.1. Synthesis of Silver Nanoparticles

A total of 10 actinobacterial strains isolated from various habitats and identified by molecular methods from previous studies (Table 1) were analysed for their potential to synthesize silver nanoparticles.

Table 1. Actinobacterial strains tested for biological synthesis of silver nanoparticles

| **Strain No** | **Isolation source** | **The most closely related species** | **Reference** |
|---|---|---|---|
| BG9H | Soil | *Streptomyces huasconensis* | Ateş, 2019 |
| BSP1 | *Lathyrus* sp. nodule | *Streptomyces albidoflavus* | Ay, 2020 |
| MCA2 | *Lathyrus* sp. nodule | *Streptomyces decoyicus* | Ay, 2020 |
| MK37H | Soil | *Streptomyces yogyakartensis* | Ateş, 2019 |
| NRH12 | Lake sediment | *Streptomyces hirsutus* | Ay, 2016 |
| NTH33 | Soil | *Streptomyces kebangsaanensis* | Ay, 2016 |
| SN42 | Soil | *Crossiella cryophila* | Mohammed, 2022 |
| SN43 | Soil | *Saccharothrix espanaensis* | Mohammed, 2022 |
| SP618 | Soil | *Streptomyces cacaoi* subsp. *cacaoi* | Mohammed, 2022 |
| YS415 | Soil | *Streptomyces bobili* | Al Yousif, 2022 |

After mixing culture broths with 2 mM $AgNO_3$ solution (1:1), the mixtures were checked every day for colour change, which is an indication for the biological synthesis of silver nanoparticles. Strain BSP1 was the most effective one in terms of incubation time and synthesis process. Thus, *Streptomyces* sp. BSP1 was selected for large scale cultivation and synthesis of silver nanoparticles (Figure 3.1).

Figure 3.1. Morphological appearance of *Streptomyces* sp. BSP1 after incubation at 28˚C in GYM medium for 19 days

### 3.2. Genome Features of *Streptomyces* sp. BSP1

The whole genome data of *Streptomyces* sp. BSP1 was downloaded from the NCBI under the accession number JAMGZL000000000. The genome data was annotated on RAST server (https://rast.nmpdr.org/rast.cgi). The genome size and DNA G+C content were determined as 7.0 Mb and 73.5%, respectively. The genome of *Streptomyces* sp. BSP1 encodes 6245 proteins and 68 RNAs. A large portion of the genome is devoted for synthesis and metabolism of amino acids and derivatives while the strain encodes 43 genes responsible for virulence, disease and defence features including resistance to antibiotics and toxic compounds. The genome of strain encodes 185 genes for cofactors, vitamins, prosthetic groups and pigments, which might be involved in synthesis of silver nanoparticles. The genome contains 53 genes related to stress response, which indicates the resilience and adaptation potential of *Streptomyces* sp. BSP1 to different environments (Figure 3.2).

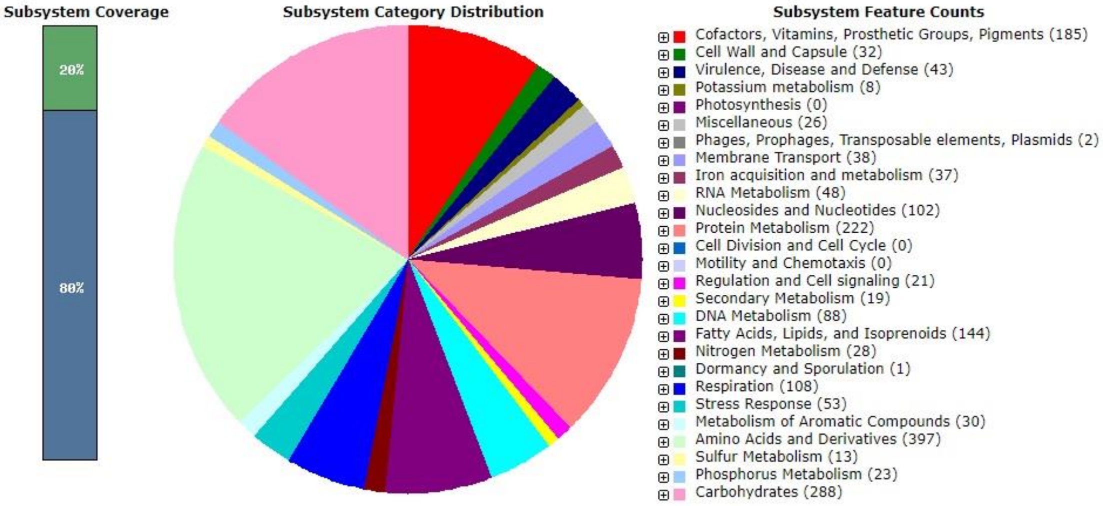

Figure 3.2. Genome annotation of *Streptomyces* sp. BSP1 as revealed by RAST server (https://rast.nmpdr.org/rast.cgi)

Bacterial silver nanoparticle synthesis might be resulted from a number of reducing agents such as reductase enzymes. Thus, silver reduction mechanisms involve an electron shuttle enzymatic silver reduction process. Nicotinamide adenine dinucleotide (NADH) generated during bacterial glycolysis and the electron transport chain by energy-generating reactions provides a cellular reducing environment suitable for the synthesis of AgNPs due to hydrogen atoms (Jha and Prasad, 2010). An important role in AgNP synthesis is ascribed to NADH-dependent enzymes, in particular nitrate reductase (Kalimuthu et al., 2008). Considering the importance of reductase enzymes in biogenic silver nanoparticle synthesis, the genome of strain *Streptomyces* sp. BSP1 was also annotated for reductase-coding genes. A total of 227 reductase genes related to nitrate/nitrite ammonification, mercury detoxification, soluble cytochromes and functionally related electron carriers, ribonucleotide reduction, coenzyme A biosynthesis, proline synthesis, heme/siroheme biosynthesis, fatty acid biosynthesis, lysine biosynthesis, purine utilization, lactate utilization, pyrimidine conversions, folate biosynthesis and anaerobic respiration were detected. However, a comprehensive analysis including molecular and chromatographic techniques must be conducted to reveal the true reducing agent in the broth of strain *Streptomyces* sp. BSP1 for silver nanoparticle synthesis mechanism.

### 3.2.1. Phylogenetic Analysis of *Streptomyces* sp. BSP1

The whole genome-based and 16S rRNA gene-based phylogenetic trees were constructed by using TYGS (https://tygs.dsmz.de/). In the 16S rRNA gene-based phylogenetic tree (Figure 3.3), *Streptomyces* sp. BSP1 was clustered together with *Streptomyces albidoflavus* DSM $40455^T$ and *Streptomyces resistomycificus* DSM $40133^T$ while the strain formed a large cluster with *Streptomyces koyangensis* VK-$A60^T$, *Streptomyces coelicolor* DSM $40233^T$, *Streptomyces limosus* NBRC $12790^T$, *Streptomyces sampsonii* NBRC $13083^T$ as well as with the type strains of *Streptomyces albidoflavus* (Figure 3.3).

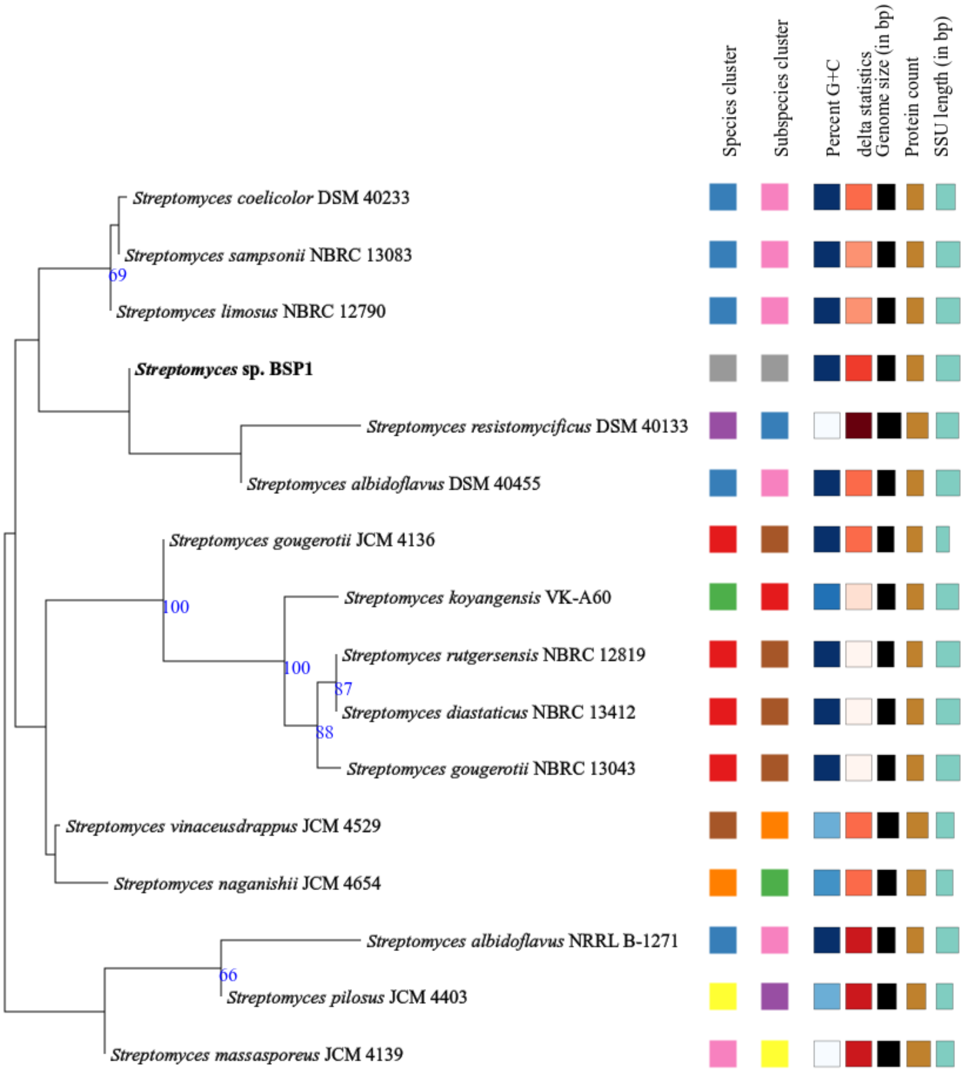

Figure 3.3. The phylogenetic tree calculated with FastME 2.1.6.1 from GBDP distances inferred from 16S rRNA gene sequences of *Streptomyces* sp. BSP1 and its most close relatives.

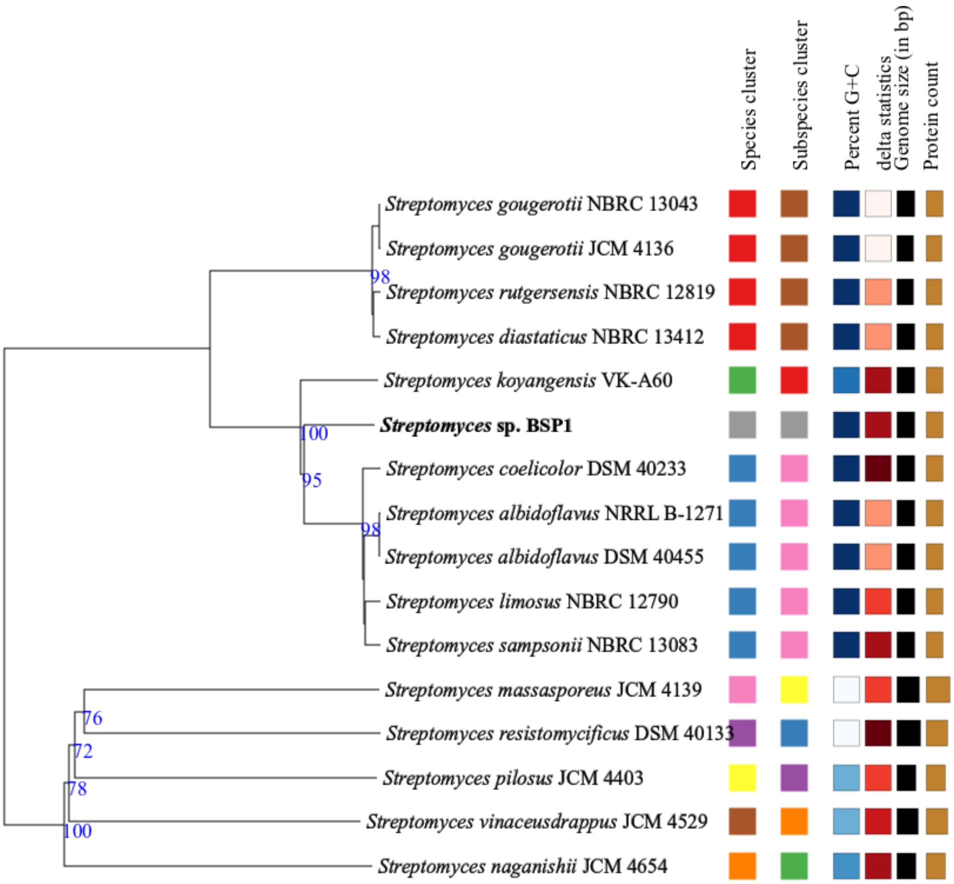

Figure 3.4. The whole genome-based tree of *Streptomyces* sp. BSP1 constructed with FastME 2.1.6.1 algorithm from GBDP distances inferred from genome data.

The digital DNA-DNA hybridization (dDDH) values between *Streptomyces* sp. BSP1 and its close phylogenetic neighbours were also determined by using TYGS. The dDDH values were lower than 70%, the threshold to delineate a novel species, which shows that strain BSP1 is a novel species within the genus *Streptomyces* (Figure 3.4).

### 3.2.2. Biosynthetic Potential of *Streptomyces* sp. BSP1 for Bioactive Secondary Metabolites

The genome of *Streptomyces* sp. BSP1 was analysed on antiSMASH server to determine bioactive secondary metabolite-coding gene clusters. The genome of *Streptomyces* sp. BSP1 have gene clusters encoding for terpenes, polyketide synthases, non-ribosomal peptide synthetases, lanthipeptides, thiopeptides and siderophores, most of which do not show considerable similarity to the gene clusters coding known secondary metabolites. The genome of *Streptomyces* sp. BSP1 have gene clusters with similarity of higher than 80% for isorenieratene, geosmin, surugamide, antimycin, desferrioxamine, SGR PTMs, ectoin, AmfS and candicidin, most of which have antimicrobial or cytotoxic activities. Considering the number and similarity levels of the gene clusters for novel metabolites, *Streptomyces* sp. BSP1 may be a good resource for novel biologically active secondary metabolites.

## 3.3. Physicochemical Characteristics of the Silver Nanoparticles Synthesized by *Streptomyces* sp. BSP1

The physicochemical characteristics of the silver nanoparticles were determined by UV-vis, FTIR, XRD and SEM-EDX analyses.

### 3.3.1. UV-vis Analysis

UV-vis spectroscopy is a practical and useful tool for physical characterization of silver nanoparticles. It is known that the optical absorption spectra of metallic nanoparticles such as silver nanoparticles are dominated by surface plasmon resonances (SPRs), which shift to longer wavelengths with increasing particle size. In addition, it is known that the extinction of silver nanoparticles primarily depends on size and shape. In general, the number of SPR peaks decreases as the symmetry of the nanoparticle increases (Guzman et al., 2012). The UV-vis spectra of the silver nanoparticles observed between 200 – 800 nm showed that the silver nanoparticles exhibited maximum absorbance peak at 439 nm, which confirms the presence of spherical silver nanoparticles.

Consistent with our results, Abd-Elhady et al. (2021) reported the extracellular synthesis of silver nanoparticles from *Streptomyces aizuneusis* ATCC 14921 with the particle size of 38.45 nm and observed that all the spectra possess main band around 403 nm.

### 3.3.2. Micromorphological and Chemical Characteristics of Silver Nanoparticles

The morphology and size characteristics of the silver nanoparticles were analysed by scanning electron microscopy technique. The SEM analysis showed that the silver nanoparticles have regular spherical shapes and their average size ranges between 7.7 nm and 15.3 nm (Figure 3.5). The ImageJ analysis using the electron microscope images showed that the particle size range between 8 nm and 22 nm, with an average particle size 13 nm, the particle size distribution is showed in (Figure 3.6). The elemental composition of silver nanoparticles was determined using the EDX method in the scanning electron microscope. The EDX analysis confirmed the presence of elemental silver by an absorption peak at 2-3 keV. The CKα, NKα, Okα, SKα and PKα peaks were also observed. These peaks are attributable to the biomolecules in the bacterial culture broth synthesizing silver nanoparticles (Figure 3.7).

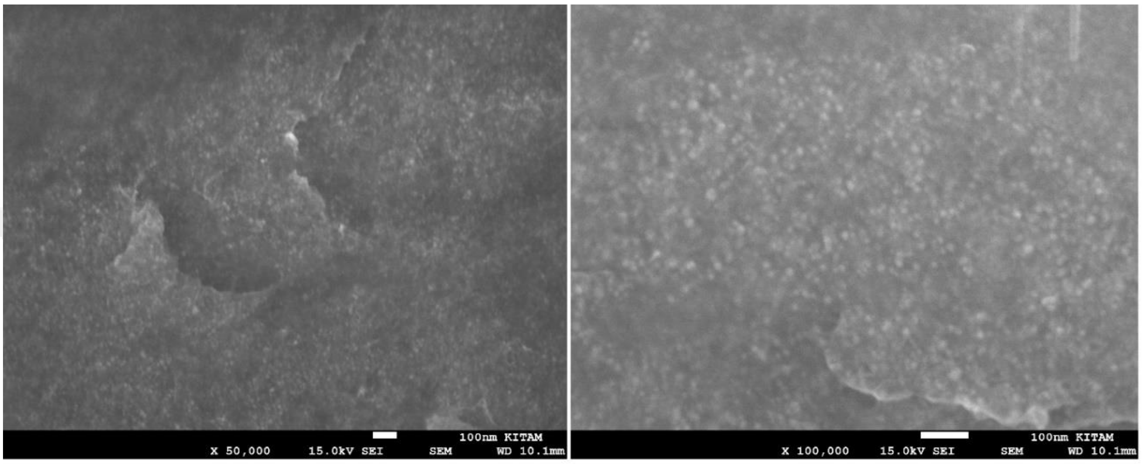

Figure 3.5. SEM image of silver nanoparticles synthesized by *Streptomyces* sp. BSP1

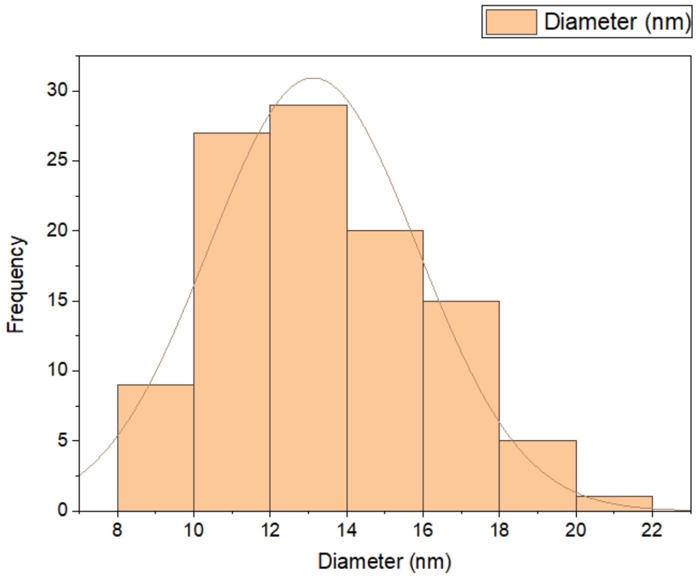

Figure 3.6. Distribution analysis of silver nanoparticles by ImageJ analysis

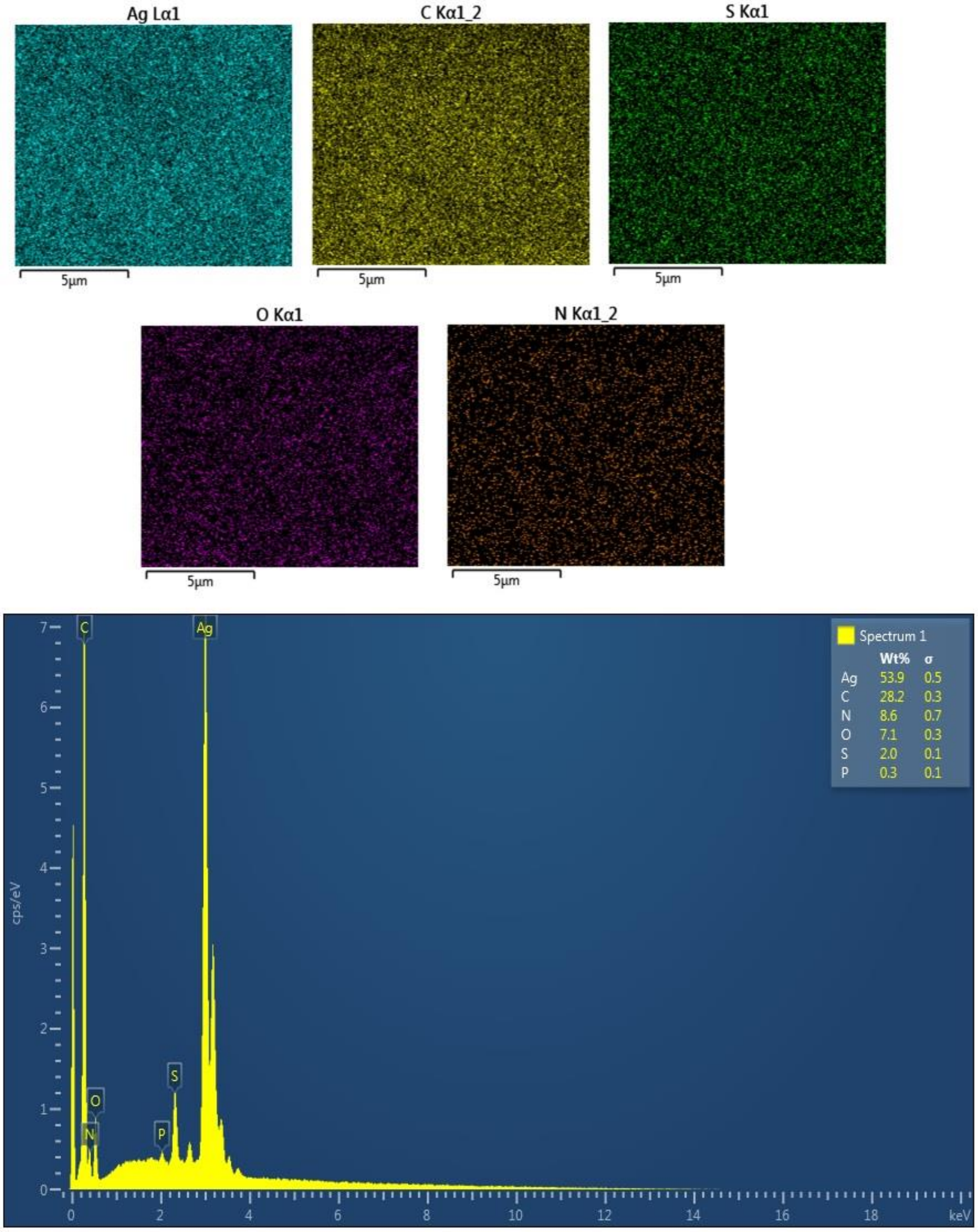

Figure 3.7. EDX analysis of silver nanoparticles synthesized by *Streptomyces* sp. BSP1

### 3.3.3. FTIR Analysis of Silver Nanoparticles

The FTIR analysis was performed to characterize the functional groups of synthesized silver nanoparticles and identify potential biomolecules responsible for the reduction of silver ions.

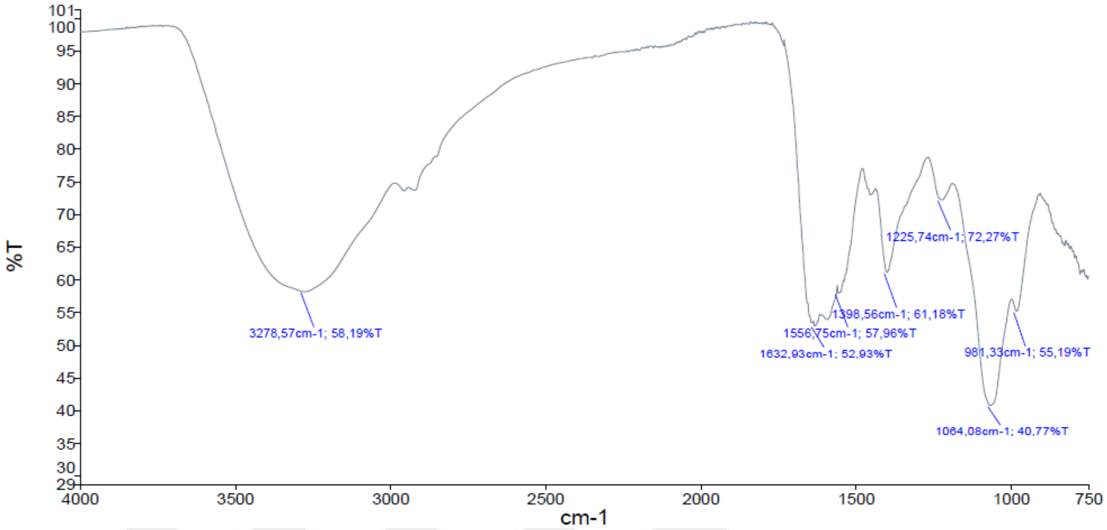

Figure 3.8. FTIR spectrum of silver nanoparticles synthesized by *Streptomyces* sp. BSP1

The silver nanoparticles showed a strong vibration at 3278.57 cm$^{-1}$ corresponding to the presence of O-H functional group while the peaks at 1632.93 cm$^{-1}$, 1556.75 cm$^{-1}$ and 1398.56 cm$^{-1}$ might result from -C=O carbonyl groups and -C=C- stretching. The other peaks observed at 1225.74 cm$^{-1}$, 1064.08 cm$^{-1}$ and 981.33 cm$^{-1}$ are corresponded to aromatic C-H in-plane bend, C-O stretch for primary alcohol and aromatic C-H in-plane bend, respectively. The FTIR results are consistent with the related literature (Bhosale et al., 2015; Nandiyanto et al., 2019) (Figure 3.8).

The FTIR spectrum identified the possible biomolecules responsible for the reduction of Ag+ ions by the cell filtrate. Most bands found in the produced AgNPs are similar to the bands obtained by Hemath Naveen et al. (2010), Yokesh Babu et al. (2014) and Sanjivkumar et al. (2019).

The peaks in the infrared (IR) spectrum could be attributed to the presence of functional groups (e.g., O-H, -C=O, -C=C-), which may have formed due to the interactions of metabolites (e.g., proteins) with the biosynthesized AgNPs. These results were consistent with those obtained by El-Naggar et al. (2017) and Saravanan et al. (2013), who reported that the functional group (or peaks) such as C=O and C-C arise from heterocyclic compounds such as proteins, which acted as capping agents for nanoparticles.

### 3.3.4. XRD Analysis

The X-ray diffraction technique was employed to analyse the crystalline nature of the silver nanoparticles synthesized by *Streptomyces* sp. BSP1. The XRD pattern of the silver nanoparticles exhibited the 2θ degrees of 18.07˚, 38.31˚, 43.81˚, 44.76˚, 64.73˚, 77.57˚, 80.83˚ and 81.51˚. The crystal planes correspond to 38.31 (111), 44.76 (200), 64.73 (222) and 77.57 (311) and the data agree well with the Joint Committee on Powder Diffraction Standards (JCPDS: 87-0597) match. The crystal peaks at 18.07, 43.81, 80.83 and 81.51 might be related to the crystalline molecules in culture broth of *Streptomyces* sp. BSP1 (Figure 3.9). The recorded peaks of AgNPs were in accordance with XRD pattern observed by Khatami et al. (2018).

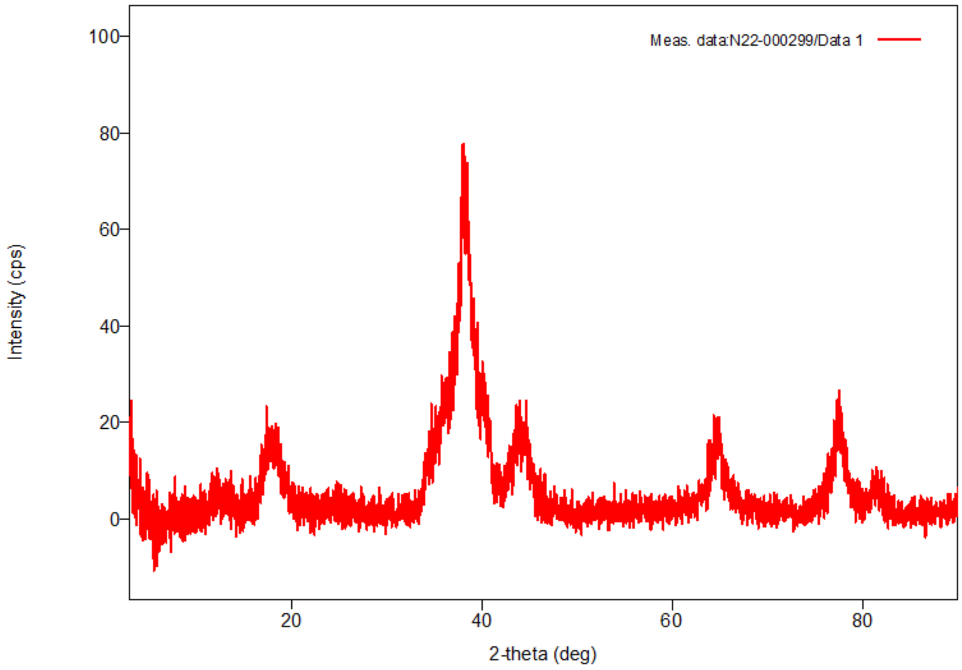

Figure 3.9. XRD pattern of the silver nanoparticles synthesized by *Streptomyces* sp. BSP1

### 3.4. Antimicrobial Activity of Silver Nanoparticles Synthesized by *Streptomyces* sp. BSP1

The antimicrobial activity analysis showed that the silver nanoparticles synthesized by *Streptomyces* sp. BSP1 have bioactivity against *Bacillus cereus* EMC15 and *Candida albicans* ATCC 10231 with inhibition zones of 14±0 mm and 15.5±0.5 mm, respectively (Figure 3.10). As revealed by the genome analysis of *Streptomyces* sp. BSP1, the genome encodes many gene clusters for novel and known bioactive metabolites. Thus, both silver nanoparticles and bioactive metabolites from culture broth of strain BSP1 may have contribution to the antimicrobial activities observed in the tests.

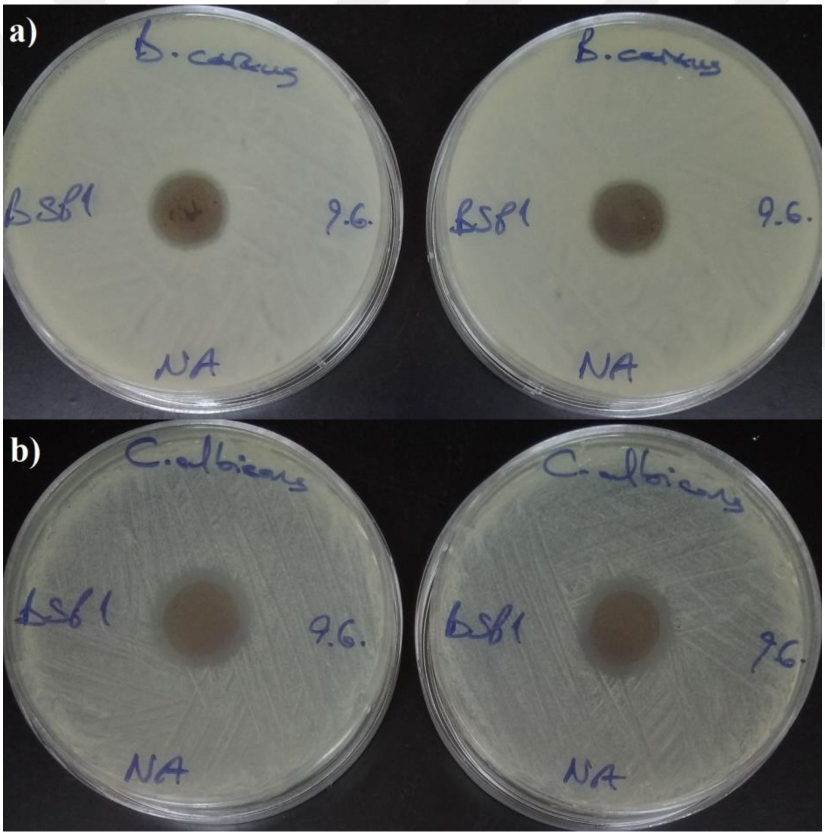

Figure 3.10. Antimicrobial activity of silver nanoparticles against *Bacillus cereus* EMC15 (a) and *Candida albicans* ATCC 10231 (b)

As a result of the antimicrobial activity test, the silver nanoparticles synthesized by *Streptomyces* sp. BSP1 can be used against infections caused by *B. cereus* and/or *C. albicans*.

### 3.5. Antioxidant Activity of Silver Nanoparticles

Antioxidant activity was assessed by examining the free radical scavenging capacity of 2,2-diphenyl-1-picrylhydrazyl (DPPH). The DPPH scavenging activity of the silver nanoparticles synthesized by *Streptomyces* sp. BSP1 was insignificant. There is a possibility that the silver nanoparticles could not scavenge oxygen radicals at all, but instead could induce the formation of reactive oxygen species.

# 4. CONCLUSION

The present study, describes the simple, non-toxic, inexpensive, clean and eco-friendly process for the extracellular biosynthesis of AgNPs mediated by an actinobacterial strain *Streptomyces* sp. BSP1, which was isolated before from a legume node in a previous study, avoiding toxic, hard chemicals and the high energy required for physicochemical synthesis. The primary clue to AgNPs synthesis was the color change in the reaction mixture from yellow to brown-black and was confirmed by UV-vis. Spectroscopy showed a maximum absorbance peak at 439 nm. The synthesized silver nanoparticles have regular spherical shapes and their average size is between 7.7 nm and 15.3 nm determined by SEM analysis. FT-IR analysis was performed to determine the biochemical functional groups involved in the capping, stabilization, and reduction of synthesized silver nanoparticles. The biogenic AgNPs produced by *Streptomyces* sp. BSP1 showed enhanced antimicrobial activity that inhibited the growth of *Bacillus cereus* EMC15 and *Candida albicans* ATCC 10231. Overall silver nanoparticles synthesized using by strain *Streptomyces* sp. BSP1 will provide an outstanding and unique basis for the submitted nanomedicine which will help prevent the spread of infectious diseases and may have significant impact on pharmaceutical industry. In addition to the biotechnological importance of silver nanoparticles synthesized by *Streptomyces* sp. BSP1, the taxonomic provenance of strain BSP1 is also another point that must be considered for future prospects. In conclusion, the silver nanoparticles with relatively small size synthesized by a novel actinobacterial species may hold high promise for biopharmaceutical applications.

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