

SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES USING MICROORGANISMS

Submitted in partial fulfilment of the requirements for the award of Bachelor of science degree in Biotechnology.

by

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INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)

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This is to certify that this Project Report is the bonafide work of S. **Afshan Banu** (40770001), R.S. **Agalya** (40770002) who carried out the project entitled **"SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES USING MICROORGANISMS."** under my / our supervision from **November 2022** to **May 2023**.

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ABSTRACT:

Silver nanoparticles (Ag NPS) have been synthesized from marine entophytic bacteria *Bacillus cereus*. Ag NPS was characterized by UV-vis absorption spectrometry (UV-vis) particle size analyzer and zeta potential. Optimisation of Ag NPS like pH, temperature and time were observed. It is of great importance in nanoscience and nanomedicine to treat and prevent serious human diseases, especially cancer therapy. In the current work, various methods to synthesize Ag NPS, such as biological, chemical and physical, as well as their properties have been described. UV-vis spectroscopy was used to characterise the reduction of the Ag ions by the supernatant of the test bacteria in the solution and the formation of Ag NPS. The UV-Vis spectrum dispersion confirmed the presence of Ag NPs. The importance of his Ag NPS in treating life-threatening diseases include cancer, along with antidiabetic, antifungal, antiviral, and antibacterial agents and their molecular mechanisms of action. The possible applications have been further discussed.

KEYWORDS: *Bacillus cereus*, Ag NPS, UV-vis spectroscopy, Particle size analyzer and zeta potential, antioxidant activity, optimization.

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LIST OF ABBREVIATION

ACRONYM	EXPANSION
ml	MILLILITRE
μl	MICRO LITRE
°C	DEGREE CELSIOUS
Ag	SILVER
NPs	NANOPARTICLES
Conc	CONCENTRATION
Temp	TEMPERATURE
Min	MINUTE
Hrs	HOURS
Org	ORGANISM
Rpm	ROTATION PER MINUTE
<i>B.cereus</i>	<i>Bacillus cereus</i>
LAF	LAMINAR AIR FLOW

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CHAPTER 1

INTRODUCTION

Brown, blue, green, blue-green, and red algae are the most common types of marine flora (i.e., Marine plants). Brown algae (kombu/kelp, wakame, and arame) are the marine floras that are most frequently used. Marine flora contains a number of minerals and offers nutraceutical benefits in addition to being a crucial part of the diet, but they cannot by themselves be considered complete foods. For bioavailable iron, they are a good source (Joseph et al, 2017). The marine fauna is extremely diverse and includes everything from tiny zooplankton to blue whales. The majority of fish and crustaceans, as well as sharks, whales, dolphins, dugongs, sea snakes, and other marine flora, spend their entire lives in the ocean. Marine fauna includes creatures like turtles, seals, sea lions, penguins, and crabs that migrate into or out of the ocean for breeding or resting. As they eat fish and other marine life, other animals like seabirds can also be categorized as marine fauna (Jailson Fulgencio de Moura et al, 2012). Even though they are considered to be part of the marine fauna, animals like sponges and coral that are attached to the seafloor are usually categorized under the environmental factor of benthic communities and habitats (Marine Fauna et al, 2016). Seaweed or marine algae is one of the most important economically valuable marine living resources that is renewable. The majority of the research over the past three decades has been on evaluating biologically active chemicals found in various seaweeds Against a variety of bacterial, fungal, and viral pathogens that are harmful to humans. Many scientists have recently developed an interest in the biosynthesis of gold and Ag NPs using a variety of plant sources, and they have produced high-quality NPs with an average size of 20 to 30 nm (C. Mohandass et al, 2013).

1. MARINE BACTERIA:

Bacteria are abundant in nature because they can adapt to any harsh environmental conditions and carry out a variety of physiological functions. Because they have such a wide range of temperature, pH, salinity, sea surface temperature, currents, precipitation regimes, and wind patterns, marine habitats are among the most unfavorable. The microorganisms living there are better suited to adapting to the unfavorable conditions as a result of the environment's ongoing change, which gives

them complex characteristic qualities of adaptability. In order to better use the bacteria isolated from marine habitats for bioremediation of heavy metals, hydrocarbons, and many other resistant chemicals and xenobiotic through biofilm formation and creation of extracellular polymeric molecules. The potential for bioremediation in several marine bacteria has been reported (Hirak R. Dash, Marine bacteria: potential candidates for enhanced bioremediation et al, 2013). Higher concentrations of marine bacteria are found in the bottom sediments than in the water, which has low levels of these organisms. The majority of these bacteria are Gram-negative and mobile (Roy M. Johnson et al,).

TABLE 1.1. TYPES OF BACTERIA:

S.NO	MARINE BACTERIA	SOURCES	pH	TEMPERATURE	NANO SIZE
1.	<i>Bacillus cereus</i>	Soil, food, and marine sponge	4.5-9.5	8°C - 55 °C	11-16 nm
2.	<i>Pseudomonas stutzeri</i> AG259	oil-contaminated soil and marine water/sediment samples.	5 – 9	4°C - 45°C	200 nm
3.	<i>Klebsiella pneumonia</i>	soil, surface waters, and plants	6 – 8	5°C - 45 °C	55 nm
4.	<i>Bacillus licheniformis</i>	soil	4.5-9.5	30°C - 50°C	50nm
5.	<i>Staphylococcus aureus</i>	soil, water and air	4 – 10	7°C - 48°C	160-80nm

1.1. BACILLUS CEREUS:

Bacillus cereus is abundant in nature and regularly isolated from soil, plants, water, and food production system niches. Recently, it was revealed that *B. cereus* could be found in a range of marine settings, including the deep sea. There are likely unreported cases of *B. cereus* food-borne infections for a variety of reasons. The use

of widely used microorganisms like *B. cereus* for the biosynthesis of Ag NPs with regulated shapes and sizes (spheres, rod triangles, wires, hexagons, cubes, and pentagons) via the intracellular and extracellular techniques should be investigated.

B. cereus is an opportunistic bacterium that also causes a number of serious non-gastrointestinal infections, including sepsis, endophthalmitis, pneumonia, and endocarditis. Several *B. cereus* exotoxins contribute to the pathogenicity of gastrointestinal infections and non-gastrointestinal infections linked to human disorders (Yan Zhao et al, 2021). *B. Cereus* is a Gram-positive aerobic or facultative anaerobic, motile, spore-forming, rod-shaped bacterium that is widely distributed environmentally. It is found in soil and marine sponge. *B. cereus* can use a wide range of substances, such as carbohydrates, proteins, peptides, and amino acids for energy and growth. The biofilm may contain metabolites, surfactants, bacteriocins, enzymes, and toxins produced by the bacteria, all of which may have an impact on the biofilm and/or its surroundings (Nada Alfryyan et al, 2022).

TABEL: 1.2. HISTORY OF NPs:

S.NO	YEAR	DISCOVERY
1.	1897	Michael faraday discovered colloidal “ruby gold”
2.	1936	An atomic resolution microscope was invented by Erwin Müller, which enabled materials to be analysed at atomic level.
3.	1947	A semiconductor transistor was discovered in 1903 by William Shockley and Walter Brattain, contributing significantly to an expansion of the scientific knowledge on semiconductor interfaces.
4.	1950	Robert Dinegar developed a process for monodisperse colloidal materials.

5.	1951	Erwin Muller pioneered the field of ion microscopes.
6.	1959	Richard Feynman suggested there's plenty of room at the bottom.
7.	1974	Taniguchi coined the term nanotechnology
8.	1981	Gerd Binnig invented a scanning tunnelling microscope allowing scientists to see individual images of atoms.
9.	1981	Russian Alexei Ekimov discovered nanocrystalline.
10.	1985	Bell labs louis brus discovered colloidal semiconductor nanocrystals
11.	1986	Gerd Binnig, Calvin Quate, and Christoph Gerber invented the atomic force microscope, which has the capability to view, measure, and manipulate materials down to fractions of a manometer in size
12.	1989	Don Eigler and Erhard Schweizer manipulated xenon atoms in the applied use of nanotechnology
13.	1990	Early nanotechnology companies began to operate.
14.	1991	Sumio Liina discovered the carbon nanotube
15.	1993	Moungi Bawndi invented a method for controlled synthesis of nanocrystals.
16.	2004	Congress established the 21st century nanotechnology research and development act.

17	2005	Erik Winfree and Paul Rothemund developed theories for DNA based computation and algorithmic self-assembly for nanocrystal growth.
18	2007-2010	DNA-robotic nanoscale assembly devices were constructed. NNI Strategic plan and the NNI environmental, health and safety research strategy.
19	2012	Nanotechnology start-ups were launched by NNI

3. NANOTECHNOLOGY:

Research in the area of nanotechnology, due to its applications across a wide range of fields, has gained considerable importance over recent years. Consequently, innovative techniques for handling and manipulation of matter at nuclear levels were formed by combining the scientific disciplines of physics, chemistry, biology or engineering. Attention is being drawn to the emphasis on materials at the nanoscale because of the more pronounced qualities that are displayed by them at this scale. In the nanoscale rather than micrometres, certain phenomena can be observed in a very significant way (Swetha et al, 2012). Nanotechnologies have grown on the back of a wide variety of efficient applications in different sectors, such as conventional chemistry techniques to medicine and environment technologies (Italo José Batista Durval 1 et al, 2021).

4. NPs:

NPs considered especially practical and effective, as they are traditionally synthesized through chemical means, are well accepted to be nano sized when their diameter is from 1 to 100 nm billionths of a meter including those in this range (Italo José Batista Durval 1 et al, 2021).in general, nanoparticles divided into four categories: inorganic, organic, polymeric NPs and carbon- based particles. All three of these group have better properties than their large counter parts. Due to their smaller size, they display properties that are enhanced, such as strength, sensitivity, high reactivity, stability,

surface area, etc. They were created using a variety of techniques that can be divided into three categories: chemical, physical, and mechanical processes. These methods have undergone a significant improvement for both research and commercial applications (M. Mohan Varma et al, 2021).

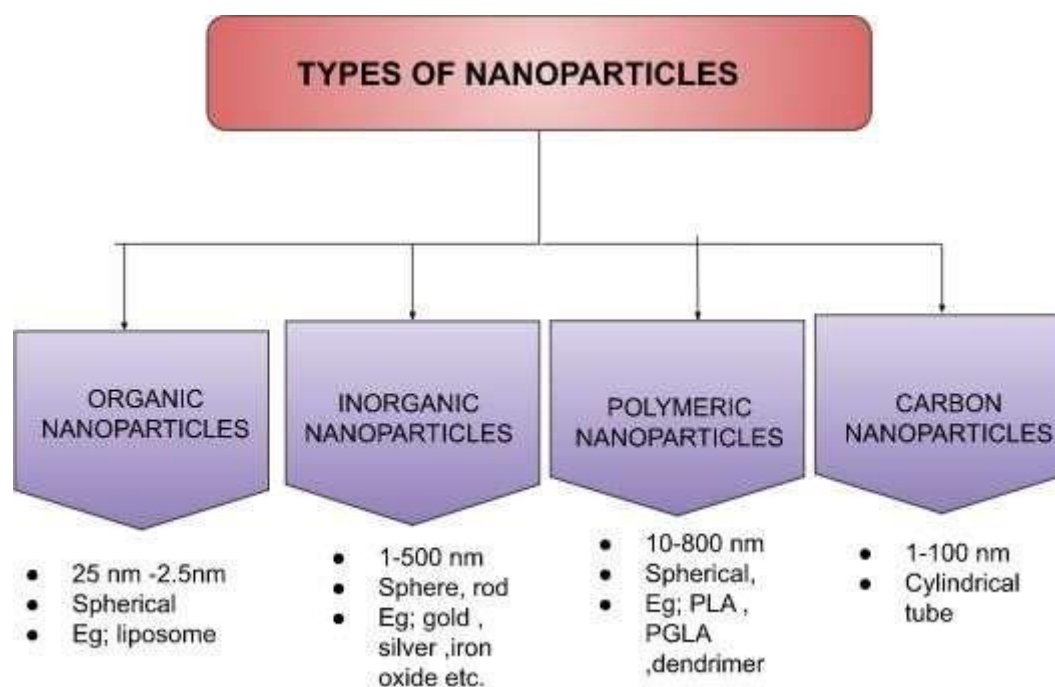


Fig 1.1: TYPES OF NPs.

1.4.1. GOLD NPs:

Due to their stability, non-toxicity, and ease of synthesis, Au NPs make an excellent study subject. They also exhibit a number of fascinating properties, such as the ability to assemble different types of particles and the quantum size effect (Guo2 et al, 2020). Due to their special optical and Physical characteristics, such as surface Plasmon oscillations for labelling, imaging, and sensing, Au NPs are widely used in many fields as preferred materials. With improved biocompatibility in disease diagnosis and therapeutics, many advances in biomedical applications have recently been made (Minakshi Das et al, 2012). With improved biocompatibility in disease diagnosis and therapeutics, many advances in biomedical applications have recently been made (Minakshi Das et al, 2012). A flexible platform for nano biological assemblies containing oligonucleotides, antibodies, and proteins is made possible by the simplicity with which Au NPs can be functionalized the design of novel biomaterials for the study

of biological systems has also made bio conjugates of Au NPs into promising candidates (Yi-Cheun Yeh et al, 2012).

2. PLATINUM NPs:

Platinum (Pt) has attracted much attention because Pt is the best catalyst that can be used for many special purposes. As a precious metal, Pt has the best catalytic activity of all pure metals, especially in fuel cells. The electro catalytic activity of Pt particles is affected by many factors such as size, distribution and synthesis method. The catalytic activity of Pt is better when nano sized Pt particles are prepared ((H. F. Aritonang et al, 2014). metal NPs platinum NPs (Pt NPs) are very important and promising because they are used in diverse fuel cells, water gas exchange reactions, electronics, petrochemicals, organic catalysis, selective oxidation of CO, automobiles, photonics, optics, biosensors and medicine (Khalida Bloch1 et al, 2021).

3. Ag NPs:

Ag NPs have been the target of researchers due to their unique properties (e.g., size and shape depending on optical, antimicrobial and electrical properties). Various preparation methods have been described for the synthesis of Ag NPs; prominent examples include laser ablation, gamma radiation, electron radiation, chemical reduction, Photochemical methods, microwave processing, and biological synthesis methods (S. Iravani et al, 2014). Ag NPs have been shown to inhibit the growth and proliferation of many bacteria such as *Bacillus cereus*, *staphylococcus aureus*, *Citrobacter koseri*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Vibrio parahaemolyticus*, and *Candida* al. biomolecules in microbial cells (Siddiqi1 et al, 2018). As well as being an important part of targeted therapy, gene delivery and detection diagnosis, they also serve as biomarkers and are used in genetic testing. In addition to this, they also contribute significantly to other sectors, such as the cosmetic industry, Agriculture, industrial industry as a whole.

1.5. METHODOLOGY:

Methods can be classified and categorized according to common approaches and differences such as Reagents and reaction conditions. Top-down versus bottom-up, green versus non-green, and conventional versus unconventional synthesis methods

are described. Top-down and bottom-up are two methods for the synthesis of metal NPs, which include chemical, physical and biological means.

1. CONVENTIONAL METHOD:

Conventional methods of synthesizing NPs are expensive, toxic and environmentally unfriendly. To overcome these problems, we use natural sources such as plants, bacteria, fungi, and biopolymers to synthesize Ag NPs. These natural resources act as reducing and capping Agents (Nazia Tarannum et al, 2019). Traditional synthetic methods include the use of citrates, borohydrides, two-phase systems (aqueous-organic), organic reducing Agents such as cyclodextrins and micelles, and polymers in the synthetic process (Erdal et al, 2018).

2. UNCONVENTIONAL METHOD:

unconventional techniques (ultrasound and microwave irradiation) offer alternative pathways for reactions via the formation of high-energy intermediates. Therefore, sonication is an alternative method for the rapid and easy synthesis of various heterocycles and fused heterocycles. This includes laser ablation, radio catalysis, metal vacuum deposition, irradiation, Photolithography, and electrodeposition (Erdal et al, 2018).

1.5.3 TOP-DOWN METHOD:

The top-down method essentially uses the material in its bulk form, and then uses specialized ablations, such as Lithography, thermal decomposition, laser ablation, mechanical milling, etching, and sputtering, to reduce the size to the nanoscale (Cheah Liang Keat et al, 2015). When processing nanostructures, the top-down method starts with macroscopic initial structures that can be controlled from the outside. Examples of the top-down approach include the creation of NPs through the mechanical grinding of bulk metals and the addition of colloidal shielding Agents (Erdal et al, 2018). A top-down manufacturing approach is a lithographic technique that uses an improved short wavelength visual light source. A major advantage of top-down technology in the manufacture of interconnect circuits is the fact that all of its parts are created and structured in an ordered fashion, requiring no further assembly. With a high degree of polish, visual Lithography was developed specifically for the production

of micro-electric chips with wavelengths reaching sub-100-nanometer levels (by conventional methods).

1.5.4. BOTTOM-UP METHOD:

The bottom-up strategy incorporates key into large elements into large structures using nanoscale physical and chemical processes. With a smaller unit size, the method produces a relevant outcome compared to the top-down method. This strategy, in which the natural forces of life harness their chemical counterparts to produce the structures, is motivated by biological systems. Examples of bottom-up techniques include decomposition, electrochemical processes and metal reduction. The synthesis methods can also be divided into green and non-green categories (Erdal et al, 2018). Bottom-up preparation of NPs is typically preferred, as it involves a homogeneous system in which catalyst synthesis nanostructures are controlled.

1.6. SYNTHESIS OF NPs:

Nanomaterials are divided into non-metallic materials and inorganic materials (TiO₂, SiO₂, ZnO₂, Al(OH)₃, Fe₂O₃, CeO₂, ZrO₂, CaO, ITO, ATO), Metals and metal alloys (Au, Ag, Pt, Pd, Cu, Fe, Ni, Co, Al, Mn, Mg), carbon-based nanomaterials (fullerene, carbon nanotube, carbon nanofibers, graphene), dendrimers and Nano polymers (polymeric nanotubes and NPs, nanowires, nano rods, nano cellulose, polymer films) and quantum dots (cadmium telluride, cadmium) selenide, cadmium-free quantum dots (Shanmuganathan¹ et al, 2019). For the synthesis of NPs, marine bio resources such as microalgae or seaweeds, microalgae (Phytoplankton), seagrass, bacteria, fungi, sponges, annelids (such as polychaetes), and mangrove plants are used. Physical, chemical, biological and mechanical methods can use to synthesis NPs. There are two mechanisms for NPs to biosynthesize: bio reduction or bio sorption. With bio-reduction, the Ag ions are transformed physiologically into more stable forms. The organism often gains from the reduction of the Ag ions in this manner. A metal ion is reduced to generate stable NPs that can be easily extracted from the sample in this redox process, while an enzyme from the organism is simultaneously oxidised. In bio sorption, the metal attaches to the living thing; often, the cell wall. Following this, NP are created as a result of an interaction with biomolecules on the cell wall (Ramya Vishwanath et al, 2021). For synthesis of Ag NPs chemical, Physical and biological methods are shown below.

1. PHYSICAL METHODS:

The most important Physical methods for the synthesis of the Ag NPs are evaporation-condensation, laser ablation, electrical irradiation, gamma irradiation, and Lithography (Erdal et al, 2018). Physically, metallic solid Ag is first vaporised in a furnace using either electrical arc discharge or ordinary heating, and then it is condensed as NP. By adjusting the thermal or ac power, and arc discharge, Ag NPs can be created with consistent particle size, narrow particle size dispersion, and uniform shape. This traditional technique often has a large energy requirement, heats up the surroundings, and takes a while to reach thermal stability. Another Physical technique for producing monodisperse Ag NPs in a polyvinyl pyrrolidone (PVP) matrix without the need of a reducing Agent is spray pyrolysis (thermal decomposition). In this procedure, an electrically heated horizontal tube reactor is first filled with an aqueous solution of AgNO_3 , dextran, or polyvinyl alcohol (PVA), which is then ultrasonically atomized and transported out of the reactor by nitrogen gas, protecting the Ag NPs from oxidation. Another method of creating Ag NPs is by directly sputtering Ag metal into a liquid media (glycerol and water).

2. CHEMICAL METHODS:

The most prevalent and straightforward strategies for making Ag NPs are chemical ones. One of the key difficulties of this process is controlling particle development to produce small, spherical Ag NPs with narrow particle size distribution. Chemical reduction using reducing Agent that are more popular for creating Ag NPs. For the reduction of Ag ions (Ag^+) in aqueous or non-aqueous solutions, a variety of reducing Agents including sodium citrate, ascorbate, sodium borohydride (NaBH_4), elemental hydrogen, polyol process, Tollens Reagent, N, N-dimethylformamide (DMF), and poly (ethylene glycol)-block copolymers are typically use (S. Iravani et al, 2014). polyvinyl pyrrolidone (PVP), polyvinyl alcohol (PVA), and ethylene glycol are the stabilizing Agent used for synthesis of Ag NPs. The toxicity of the employed solvents has a long-lasting effect, endangering humans after the NPS have been created as well. On the surface of the synthesised Ag NPs, chemical residue from the solvent is frequently still present. A glimpse of the health risk connected with the primary chemicals used during the chemical synthesis of Ag NPs is provided by the fact that these NPs are particularly

harmful when used for drug delivery, antimicrobial action, or any other use that necessitates the NPs to be inserted inside the human body (Ramya Vishwanath et al, 2021).

1.6.3. BIOLOGICAL METHODS:

Biological methods can be used to create well-defined NPs. Some important factors that must be considered when creating these particles include the types of organisms used, their inheritable and genetic properties, the conditions under which they will grow, and the enzymes and materials used in the reaction. By adjusting a few key factors, such as the substrate concentration, pH, light, temperature, buffer strength, electron donor (like glucose or fructose), and biomass and substrate concentrations, the size and shape of the NPs can be controlled (S. Iravani et al, 2014). It can be synthesized by bacteria, fungi, plant extract, viruses and algae. The main Advantages of biological synthesis are the safety of the procedure and the quality of the produced NPs. We can be certain that the product produced will be free of any contamination because this approach uses only benign substances to enable the creation of Ag NPs. This has a negligible effect on people's health, especially considering the many different medical uses for these NPs. In addition to this, biological procedures frequently produce NPs with a higher yield than physicochemical methods and that are homogeneous in size and shape (Ramya Vishwanath et al, 2021).

1.7. BACTERIA:

The synthesis of Ag NPs using bacteria involves growing the bacteria in a pot, then contact of the bacteria or their metabolites with a AgNO_3 solution (Lima* et al, 2019). The different types of bacteria in the test were *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumonia*, *Lactobacillus acidophilus*, *Staphylococcus cereus*, and *Pseudomonas aeruginosa*. *Candida albicans* was also included (Ahmad R. Shahverdi et al, 2007). Bacteria use a variety of methods to make tiny metal particles. In some cases, they use enzymes that are located inside their cells. In other cases, they collect tiny metal particles from the environment, and then use them to make new particles. In either case, the metal particles accumulate in different parts of the bacteria cells. Microscopic analysis of these cells has shown that metal particles are often found in the periplasmic, cytoplasmic, and cell wall components (Divyanshi Garg et al, 2020). There are two

ways that microorganisms can produce Ag NPs: intracellularly and extracellularly. In the intracellular approach, Ag ions build up inside the cells, inducing nucleation, and the production of Ag NPs continues as the microbes develop. Bacteria used for intracellular production have negatively charged cell walls that draw in positively charged Ag ions. Numerous enzymes that can decrease the Ag ions to create stable NPS are abundant in the bacterial cell wall. The secretions of microorganisms that contain reducing Agents are isolated and collected using the extracellular approach in the form of a filtrate. Synthesis is done using the filtrate that contains the biomolecules. Numerous investigations have validated the function of prosthetic groups and cofactors in the extracellular mode (Ramya Vishwanath et al, 2021).

8. FUNGI:

Fungi are organisms that can produce NPs. These particles can be created by both extracellular and intracellular means. When fungi are under stress, they produce enzymes and proteins to counteract the stressors. These particles can be created from both extracellular and intracellular materials (Divyanshi Garg et al, 2020). Fungi are attractive tools for the biosynthesis of Ag NPs due to their high metal tolerance and ease of handling. They also secrete large amounts of extracellular proteins that contribute to nanoparticle stability. The advantage of fungal cultures over bacterial systems is that they provide good biomass production and do not require additional steps to extract the filtrate. Compared to plant synthesis, fungal Hyphal masses are more resistant to movement and pressure, making them suitable for large-scale synthesis. Furthermore, by adjusting culture conditions such as time, temperature, pH and amount of biomass, fungal metabolism can be manipulated to obtain NPs with desired properties such as specific size and morphology (Lima* et al, 2019).

9. ALGAE:

The synthesis of Ag NPs in algae is of particular interest due to the high ability of algae to adsorb metals and reduce metal ions. Algae are very widespread organisms, and their availability is high; an additional advantage is their growth under laboratory conditions. These organisms can aid in large-scale, inexpensive production. They are unicellular or multicellular organisms that inhabit a variety of habitats, including freshwater, seawater, and wet rock surfaces. There are two different categories of

algae: microalgae (microscopic) and macroalgae (macroscopic). They play an important role in applications such as pharmaceuticals, forestry, aquaculture and cosmetics. They are important sources of several commercial products, such as natural dyes and biofuels. Algae are used for the synthesis of NPs because of their high potential for metal enrichment, ease of handling and cultivation, ability to grow at low temperatures, and low toxicity to the environment. Green algae, brown algae, cyanophytes, and red algae are the most common algae used for the synthesis of Ag NPs (Deeksha Chugh et al, 2021).

10. PLANT:

The synthesis of NPs using plants is very inexpensive and can therefore be used as a cheap and valuable alternative to large-scale NPs production. *Camellia sinensis* (green tea) extract has been used as a reducing and stabilizing Agent in the biosynthesis of Ag NPs. The Phenolic acid-like biomolecules (e.g., caffeine and Theophylline) in it appeared to be responsible for the formation and stabilization of Ag NPs. Black tea leaf extracts have also been used to create Ag NPs. The NPs were stable and had various shapes such as Spheres, trapezoids, prisms, and rods. It seems that Polyphenols' and flavonoids are responsible for the biosynthesis of these NPs (S. Iravani et al, 2014). Using Plants to Produce Ag work their fast, Eco-friendly, non-pathogenic, economical protocol and Enables one-step technology for biosynthetic processes Reduction and stabilization of Ag ions by incorporation of biomolecules such as proteins, enzymes, amino acids, polysaccharides, alkaloids, phenols, tannins, Saponins, terpenoids, vitamins Already established with medicinal botanical extracts environmentally friendly and chemically complex Structure (Sivakumar et al, 2021).

11. MARINE BACTERIA BASED SYNTHESIS OF Ag NPs:

Ag NPs have been synthesized from marine matter Filtered bacterial isolates of culture (MER1) and are considered to be ecological nano factories responding to many biotechnologies request. The synthesized NPs were found to be Spherical, 20-40 nm in size. The energy dispersion spectra of the nanoparticle dispersion confirmed the presence of elemental Ag. Ag NPs have been shown to have antimicrobial activity Against various pathogenic bacteria such as *Escherichia coli*, *Pseudomonas*

aeruginosa, *staphylococcus aureus*, *Salmonella typhi*, and *Klebsiella pneumonia* (Swetha et al, 2012). they are Ag NPs with a wide range of applications in various fields, including antimicrobials, paint preservatives and Cosmetics. Properties of Ag ions exposed to the microbial strain and reduced the relationship between AgNO₃ and Ag NPs has been confirmed using a Visible Spectrophotometer. Toxicity studies the use of marine bacteria is good approach to making green products affordable Ag NPs. Many NPS on human pathogens open the door for a new range of antibacterial action (P Shivakrishna et al, 2013). Cyanobacteria produce Ag NPs *Clostridium* carried out the biosynthesis of cadmium NPS in *Plectonema boryanum*. *Actinobacter sp. Shewanella oneidensis* was reported to produce thermoaceticum, Magnetite NPS, and uranium NPs. The use of nanocrystal line Ag dressings, creams, and gels to effectively reduce bacterial infections in chronic wounds is just one example of how important Ag NPs have been in many different fields. The poly vinyl nano-fibre-containing Ag NPs exhibits effective antibacterial properties as a wound dressing. When tested, Ag NPs reportedly demonstrated improved ability to heal wounds, improved cosmetic appearance, and scar-less healing (Rajeshkumar S et al, 2013).

1.12. SECONDARY METABOLITES OF MARINE BACTERIA:

A wealth of compounds is produced by marine derived secondary metabolites with intriguing structures. Although most structural features correspond Some marine organisms, such as halogen, isonitrile, isothiocyanate or areothiocyanate, are very similar to those found in terrestrial organisms. Proxy substituents or aromatic heterocyclic that are very condensed. Many composites have powerful bioactivities like poisons or healing agents. In particular, they may help to protect animals from predators, help competitors succeed, or help animals mark their territory (Rinehart, 1992). Biosynthetic gene clusters (BGCs) are groups of genes that work together to make a molecule. Some of the largest BGCs include non-ribosomal peptide synthetases (NRPSs) and polyketide synthases (PKSs), which are responsible for building many of the antibiotics and antifungals in the world. NRPSs have a condensation (CD) and adenylation (AD) domain, and PKSs have a ketosynthase (KS) domain. These domains can be used to identify new BGCs, and their abundances can be used as a proxy. for biosynthetic potential (Allison M. Sharrar et al, 2020). Because of their abundant biodiversity and genetic capacity to synthesise distinctive secondary metabolites, marine microorganisms are an important source for the discovery and

development of novel physiologically active chemicals. This collection of metabolites, which are abundant in marine bacteria, fungi, archaea, and other microbes, is crucial to the survival of those species. These complex macromolecules produced by marine prokaryotes contain substances with a variety of biological activities, such as antibacterial, antifungal, antiviral, and antiproliferative Agents, exotoxins, metal carriers, hormones, immunomodulators, pigments, and enzyme inhibitors. (Gokulan. K et al, 2014). Particularly, marine bacteria's secondary metabolites have proven to be a significant source of organic compounds with novel structures and strong antibiotic action. For instance, more than 5000 antibiotics related to secondary metabolites have been identified among actinomycetes, which represent the most significant source of biologically active natural products for clinical or pharmaceutical use. These antibiotics include conventional antibiotics discovered in the 1950s and 1960s as well as antibiotics. These bacteria have the potential to manufacture up to 150,000 distinct chemical antibacterial compounds, according to predictions. (Wang et al.2014). Secondary metabolites are a class of structurally diverse, complex, low-molecular-weight bioactive molecules that are now recognised by modern research. The active stage of these compounds' synthesis in microorganisms has been discovered to take place at the end of the exponential and the start of the stationary phases of their growth. The genes involved in the manufacture of secondary metabolites are arranged in a few clusters and their production is triggered by nutrient deficiency and unfavourable habitat circumstances (Pinu et al .2017, Baral et al.2018). A wide range of molecules, ranging from proteins to various low molecular weight compounds such as terpenoids, alkaloids, amino acids, alcoholic compounds, Polyphenols (catechin, flavones, taxifolin, procyanidins of various chain lengths formed by catechin and epicatechin units, and phenolic acids), glutathione, polysaccharides, antioxidants, organic acids (ascorbic, oxalic, malic, tartaric, and protocatechuic acid), quinones etc., have been reported to play a role in the green synthesis of NPs. The participation of sugars, terpenoids, polyphenols, alkaloids, phenolic acids, and proteins in the reduction of metal ions into NPs and in supporting their subsequent stability has also been postulated (Gregory Marslin, 2018).

13. APPLICATION OF Ag NPs:

1. ANTIBACTERIAL ACTIVITY:

The antibacterial activity of the Ag NPs is extraordinarily high. The two bacteria with the highest antibacterial activity were *staphylococcus aureus* and *Bacillus subtilis*. According to an article, Ag NPs antibacterial activity was tested Against a particular bacterial strain, and the results revealed that Ag NPs increased the rate at 31.25 M/mL. *Escherichia coli* and *Pseudomonas aeruginosa* were the microorganisms that were utilised. The fundamental idea is to make gram-negative bacteria more permeable by creating hydroxyl radicals (OH⁺), which eventually lead to cell death. Size is important; the more surface area there is, the more attachment and penetration that might result in harm. Ag NPs were created by using the leaf *Berberis vulgaris*, and their antibacterial activity was tested using *gentamicin* and *streptomycin*. The killing of microbes may be attributed to the Ag NPs ' ability to continuously release Ag. Ag ions can cling to the cell wall and cytoplasmic membrane due to electrostatic attraction and affinity to Sulphur proteins. The bacterial envelope may be damaged as a result of the adhered ions increasing the cytoplasmic membrane's permeability. Respiratory enzymes may be inactivated following the uptake of free Ag ions into cells, leading to the production of reactive oxygen species but not adenosine triphosphate. Deoxyribonucleic acid (DNA) modification and cell membrane disruption can both be caused by reactive oxygen species (Iris Xiaoxue Yin et al, 2020).

2. ANTIFUNGAL ACTIVITY:

In this study, Ag NPs ' ability to continuously release was used to slow down the candid strain's development. Ag NPs are able to combat fungal infections at low concentrations. An Ag NPs with a thin shell is capable of damaging the cell wall of a *Candida* strain, which will cause the infection to cease. Free radicals were released as part of the mechanism, which ultimately resulted in the death of the cells. This article talks about a study which discussed the creation of graphene, iron, copper, and Ag NPS as well as their antifungal properties. According to the data obtained using the fungi *Aspergillus flavus* and *Aspergillus parasiticus*, Ag NPs were found to be the most effective antifungal compounds followed by Cu-NPs and Fe-NPs. During the interaction between the NPS and the cell, the NPS prevent the cell from replicating, which in turn is responsible for the replication of the cell.

3. ANTICANCER ACTIVITY:

Ag NPs has a strong anticancer potential because it inhibits the mitochondrial respiratory chain, boosts the production of reactive oxygen species (ROS), and ultimately causes DNA damage and cancer cell death. The treatment with camptothecin and Ag NPs significantly raises the levels of cancer cells, according to research by (Yu-Guo et al ,2018, Yuan et al. 2018). In comparison to a single treatment, it increases oxidative stress markers and decreases anti-oxidative stress markers. These findings collectively suggested that camptothecin and Ag NPs cause cell death by altering the permeability of the mitochondrial membrane and activating caspase. Increased ROS formation and antioxidant depletion appear to be related to the synergistic cytotoxicity and apoptosis effect. Certainly, combining CPT with Ag NPs has an advantage over other methods for treating cervical cancer (Sunil T. GalatAge et al, 2021). Many cancers are unresponsive to platinum-containing drugs, and there are many toxic side effects, including gastrointestinal and haematological toxicity. In addition, many tumour cells have inherited or acquired resistance other platinum formers and cisplatin. As a result, current cancer research has been devoted to the discovery of new transition metal compounds. (Wafa I Abdel Fattah et al, 2018).

4. ANTIVIRAL ACTIVITY:

Ag NPs have been shown to have antiviral activity Against HIV-1 at non-cytotoxic concentrations, but the mechanism underlying this activity is still not completely understood. After infection with the H3N2 influenza virus, the study from intranasal administration of Ag NPs in mice showed increased survival, decreased lung viral titer levels, minor pathologic lesions in lung disease, and a remarkable survival benefit, indicating that Ag NPs played a significant role in mice survival. Based on size and zeta potential, biologically produced Ag NPS inhibited the viability of human para influenza virus type 3 and the herpes simplex virus (HSV) types 1 and 2 (Sunil T. Galatage et al, 2021). Using indicator cells in which infection was measured using a luciferase-based assay, Ag NPs of 30-50 nm was tested Against a panel of HIV-1 isolates (Humberto H Lara et al, 2010).

5. ANTIOXIDANT ACTIVITY:

The synthetic Ag NPs has the ability to scavenge superoxide, hydrogen peroxide, hydroxyl radicals, and antioxidants. Ag NPs ' functional groups on the surface are what

cause this activity to happen (Anand Kumar Keshari a et al, 2020). Ag NPs ' substantial antioxidant potential was assessed using a DPPH radical scavenging assay with an IC₅₀ of 30.04 g/m (Yugal K. Mohanta^{1*} et al, 2017). Methanol was used as a blank solution and DPPH was used as the control (all the reagents were used except for the sample). The antioxidant activity was evaluated using DPPH radical scavenging assay, determination of total reductive potential, total phenolic content (TPC) and total flavonoids content (TFC) of the synthesized Ag NPs. SEM analysis revealed that the size of the synthesized Ag NPS ranged from 50-70 nm with maximum UV-vis spectroscopy absorbance at 413 nm. DPPH radical scavenging activity, reducing power, total phenolic and total flavonoid contents of the synthesized Ag NPs increased in a dose dependent manner as compared to ascorbic acid the standard reference used (AO Akintola^{1*}et al, 2020).

1.13.5 ANTI –INFLAMMATORY ACTIVITY:

Although Ag NPs are known for their antimicrobial properties, the anti-inflammatory response is still in its infancy. Nanocrystal line Ag (NP32101) was administered intra colonially or orally to rats, and they displayed significantly less colonic inflammation. Ag NPs demonstrated dose-dependent rapid healing and improved cosmetic appearance. A small amount of ethyl acetate and petroleum ether were used in the extraction process to create Ag NPs, which have strong cyclooxygenase-2 inhibitory properties. Therefore, the anti-inflammatory activity of the Ag NPs produced by petroleum ether extraction is increased when one adds a natural extract with anti-inflammatory activity to them. *NepHthys sp.*, a soft coral, was recently extracted by some scientists. which already had anti-inflammatory properties and removed the Ag (Sunil T. Galatage et al, 2021).

1.13.6. ANTIDIABTIC ACTIVITY:

Ag NPs synthesis via *Tephrosia tinctoria* stem extracts was assessed for blood sugar level control. Ag NPs neutralized free radicals, decreased the activity of the enzymes (glucosidase and amylase) responsible for the hydrolysis of complex carbohydrates, and increased the rate at which glucose is consumed. The Ag NPs from *Ananascomosus (L.)* have demonstrated some encouraging anti-diabetic activity. in a manner dependent on dose. The stomach's -glucosidase enzyme is inhibited by AC-Ag NPs. which is beneficial for diabetic patients not taking insulin. Additionally, theAg

NPs made with *Argyrea nervosa* leaf extract demonstrated strong anti-diabetic properties. They inhibit mainly enzymes that digest the carbohydrates into monosaccharide and reduce blood glucose level (Sunil T. Galatage et al, 2021). Diabetes is a condition where a person has too much sugar in their blood. This can lead to problems such as heart problems, blindness, and kidney problems. There are two types of diabetes - Type I and Type II. Type I diabetes is when the pancreas doesn't produce enough insulin, while Type II diabetes is when the body's insulin isn't used effectively. Gestational diabetes is when a person has high blood sugar levels during their pregnancy. Anti-diabetic medications help to lower blood sugar levels by suppressing the enzymes that cause it. However, these medications have some side effects, such as diarrhoea, bloating, and swelling (Ramya P, et al).

7. *DIAGNOSTIC, BIOSENSOR, AND GENE THERAPY:*

NPs are superior to today's treatments because they can be designed to have specific properties or in specific ways. They are useful for cell imaging. Ag plays an important role in imaging systems due to its strong and sharp Plasmon resonance. Ag biosensor is currently used as a powerful tool to detect cytochrome P53 in head and neck squamous cell carcinoma. Due to their colorimetric sensor properties, Ag NPs are suitable for detecting heavy metal ions of nickel, cobalt and mercury along with trace amounts of sulphides. Among all kinds of Ag NPs, especially triangular Ag NPs have higher anisotropy and lightning rod effect, so the Plasmon sensor or Plasmon detector used to detect mercury ions in solution widely used in the production of Ag NPs are also used to develop an electrochemical sensor used to detect the common herbicide atrazine. On the other hand, the in-situ growth and development of Ag NPs on polydopamine-labelled filter paper is responsible for the rapid collection and detection of green malachite residues (Sunil T. Galatage et al, 2021)

8. *FUTURE SCOPE OF Ag NPs:*

Ag NPs ' significance in medicine is well known. Ag NPs are widely employed in a variety of fields, mostly in the medical and biological fields. Ag NPs are frequently employed as antibacterial coatings in medical devices such as cardiovascular implants, wound dressings, catheters, orthopaedic implants, dental composites, nano-bio sensing, and Agricultural engineering (Lin et al., 2011; Sumitha et al., 2019). Various infectious diseases brought on by microorganisms have been significantly

inhibited by Ag NPs. Cancer patients are being treated with the Ayurveda plant *F. indica* from India. Diagnostic and therapeutic applications of Ag NPs in medicine can be distinguished from one another. A non-invasive method of cancer screening is Surface Enhanced Raman Spectroscopy (SERS), which is based on Ag NPs. This method of cancer diagnosis will unavoidably be used in the near future. According to (Sumitha et al.2011), Ag NPs show a good inhibitory impact Against both Gram-positive and Gram-negative bacteria in the context of biomedical applications. The production of NPs may serve as a future direction in biomedical nanotechnology for the development of antibacterial substances, it may be concluded from the foregoing discussion.

CHAPTER-2

2.1. REVIEW OF LITERATURE:

Ag and Ag salts have been used for as long as there has been human civilization, but the creation of Ag NPs (Ag NPs) has only lately come to light. They have especially been employed as antibacterial, antifungal, and antioxidants in Agriculture and medicine. It has been demonstrated that Ag NPs arrest the growth and multiplication of many bacteria such as *Bacillus cereus*, *staphylococcus aureus*, *Citrobacter koseri*, *Salmonella typhii*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumonia*, *Vibrio parahaemolyticus* and fungus *Candida albicans* by binding Ag/Ag⁺ with the biomolecules present in the microbial cells. Ag NPs may generate free radicals and reactive oxygen species, which lead to apoptosis and cell death by blocking cell replication. Ag NPs permeate into cells and rupture the cell wall because they are smaller than microorganisms, as demonstrated by SEM and TEM images of the suspension containing NPs and pathogens. Additionally, it has been demonstrated that smaller NPs are more poisonous than larger ones. Ag NPs are also utilised in packaging to stop germs from contaminating food goods. Ag NPs ' toxicity is influenced by the size, concentration, pH of the media, and length of pathogen exposure (Siddiqi et al .2018). Against a variety of bacteria, Ag NPs have demonstrated strong bactericidal capabilities (Wei L et al 2018). They are produced from a variety of angles, frequently in order to examine their morphology or other physical traits. Though they did so unintentionally, several authors have mistakenly employed the chemical approach (Zaheer Z et al 2012; Rafiuddin et al ;2012) for green synthesis. Ag NPs are environmentally beneficial due to their use in electronics, catalysis, medicines, and limiting the growth of microorganisms in biological systems (Lokina S et al. 2014) Although numerous reviews on the creation and characterisation of Ag NPs have been published, there are very few studies on their environmentally friendly production, biocidal capabilities, and mechanism of action (Ahamed M et al. 2010).

2.2 TYPES OF SYNTHESIS:

Metallic NPS are often created using one of two techniques: "top-down" (slicing or sequential cutting of a bulk material to obtain nano-sized particle) or "bottom-up" (building a material from the bottom up: atom by atom, molecule by molecule, or cluster by cluster). When creating NPs in a homogeneous system, the "bottom-up" method is typically preferable since it uses catalysts (such as enzymes and reducing Agents) to create nanostructures that they themselves can control. The size reduction to the nanoscale is accomplished by specialised ablations, such as thermal decomposition, mechanical grinding, etching, cutting, and sputtering, but the "top-down" technique often works with the material in its bulk form. Surface structural flaws are the fundamental drawback of the top-down strategy. Metallic NPs' physical characteristics and surface chemistry are significantly impacted by such imperfections. For the synthesis of Ag NPs, a variety of techniques are available, including chemical, Physical and biological approaches. (Zhang Q et al. 2011).

Chemical reduction, electrochemical, irradiation-assisted chemical, and pyrolysis processes are different types of chemical synthesis. Metal precursors, reducing Agents, and stabilising or capping Agents are necessary for the synthesis of Ag NPs in solution. Ascorbic acid, alcohol, borohydride, sodium citrate, and hydrazine compounds are often utilised reducing Agents. (Zhang W, Qiao X, Chen J.,2007). According to Sotiriou and Pratsinis (Sotiriou GA, and Pratsinis SE., 2010), flame aerosol technology was used to create the Ag NPs supported on nanostructured SiO₂, allowing for precise control of the Ag concentration and size. Additionally, flame spray pyrolysis was used to create Ag /silica NPS with a rather limited size distribution (Sotiriou GA et al .2011). Physical procedures, however, typically process quickly and don't require dangerous or highly reactive chemicals. Arc discharge (Tien DC et al. 2008), physical vapour condensation (Abou El-Nour KMM, 2010) the energy ball milling method (Wright R et al. 2011) and direct current Magnetron sputtering (Asanithi P et al. 2012) are a few examples of these techniques. Physical approaches have an additional benefit over chemical methods because Ag NPs have a restricted size distribution, while the primary drawbacks include high energy consumption (Asanithi P et al. 2012). Considering the numerous benefits that biological synthesis of Ag NPs from herbal extract and/or microorganisms provides over chemical and Physical techniques of synthesis, this method has been proposed as an alternative.

Additionally, it is generally known that these approaches are straightforward, affordable, environmentally benign, and simple to scale up for high yields and/or production. In the field of nanotechnology, the biosynthesis of metal and metal oxide NPs utilising biological Agents such as bacteria, fungi, yeast, plant, and algae extracts has grown in popularity. (Siddiqi, K.S., Husen, A. & Rao et al,2018).

2.3 BACTERIAL SYNTHESIS OF Ag NPs:

The possibility of producing Ag NPs using bacteria has recently come to light (Gopinathan P, Ashok AM, Selvakumar. et al 2013). To create Ag NPs inside the cells, for instance, *Pseudomonas stutzeri* AG259 isolated from an Ag mine was utilised (Klaus T, Joerger R, Olsson E, Granqvist CG et al. ,1999). Additionally, a number of gram positive and gram-negative bacterial strains, including *A. calcoaceticus*, *B. amyloliquefaciens*, *B. flexus*, *B. megaterium*, and *S. aureus*, have been employed to produce Ag NPs both extracellularly and intracellularly (Nanda A, Saravanan et al,2009). The shapes of these Ag NPs include spheres, discs, cuboids, hexagons, and triangles. Cells, aqueous cell-free extract, or culture supernatant have all been used to create them. The extracellular biogenesis of Ag NPs (5-50 nm). (Saifuddin N, Wong CW, Yasumira AAN et al,2009) by combining *B. subtilis* culture supernatant with microwave irradiation in water. (Shahverdi AR, Minaeian S, Shahverdi HR, Jamalifar H, Nohi AA et al,2007), *K. pneumonia*, *E. coli*, and *Enterobacter cloacae* culture supernatants were used to quickly biosynthesise Ag NPs (within 5 min). In the presence of aqueous solutions of Ag⁺ ions. (Saravanan M, Venu AK, Barik SK et al,2011) also reported an extracellular production of Ag NPs using *B. megaterium* culture supernatant. The combination of a *Bacillus flexus* group bacterial strain S-27 and 1mM AgNO₃ in aqueous medium led to the rapid production of Ag NPs. The colourless supernatant solution changed from colourless to yellow and then brown. As a result of the Ag NPs surface Plasmon resonance (SPR), their UV-vis spectroscopy showed a strong peak at 420 nm. Although their progressive deterioration cannot be stopped, anisotropic NPs of 12 and 65 nm size were stable in the dark for 5 months at ambient temperature. They had a face-centred cubic structure and were crystalline. Gram positive and gram-negative bacteria that are resistant to many drugs were discovered to be sensitive to these NPs. The concentration of the reacting elements determines the intensity of the colour and the speed of the reaction. (Priyadarshini S, Gopinath V, Meera Priyadarshini N, Mubarak Ali D, Velusamy P. et al 2013).

2.4 FUNGAL SYNTHESIS OF Ag NPs:

It has been widely studied how pathogenic and non-pathogenic fungi produce Ag NPs (Durán N, Priscyla D, Marcato PD, Alves O, De Souza G, Esposito E et al, 2005). According to one study, when fungi are present, Ag ions are reduced extracellularly to produce stable Ag NPs in water (Kathiresan K, Manivannan S, Nabeal MA, Dhivya B, 2009). The extracellular production of Ag NPs by the thermophilic fungus *Hemioia sp.* (Syed A, Saraswati S, Kundu GC, Ahmad A et al., 2010). In an Erlenmeyer flask set at 50 °C with 100 mL of a 1 mM AgNO₃ solution suspended in it, the mixture was shaken for 96 hours at pH 9 while being watched for any colour changes. Due to the creation of Ag NPs, the solution's colour changed from yellow to brown (Ahmad A, Mukherjee P, Senapati S, Mandal D, Khan MI, Kumar R, Sastry M. et al, 2003). The extracellular production of Ag NPs from *Humicola sp.* is a straightforward procedure. A TEM Image revealed evenly distributed NPs, the majority of which were spherical and ranged in size from 5 to 25 nm. They have a face-centred cubic structure and are crystalline. Peaks at 1644 and 1523 cm⁻¹ in the IR spectroscopy of the Ag NPs suspension were attributed to the amide I and II bands of protein, which correspond to the -C=O and N-H lengths, respectively. (Chwalibog A, Sawosz E, Hotowy A, Szeliga J, Mitura S, Mitura K, Grodzik M, Orlowski P, Sokolowska, et al 2010).

The production of Ag NPs from the yellow exotic oyster mushroom *Pleurotus cornucopiae var. citrinopileatus* (Owaied MN, Raman J, Lakshmanan H, Al-Saeedi SSS, Sabaratnam V, Abed IA et al., 2015). The dried basidiocarps were pulverised, then the supernatant was boiled in water and freeze dried. At 25 °C, 1 mM AgNO₃ was mixed with various hot water extract quantities of this lyophilized powder, and the mixture was then incubated for 24, 48, and 72 hours. An absorption peak at 420 and 450 nm in the UV-vis spectroscopy range was visible as the colour changed from yellow to yellowish brown, which is a hallmark of spherical Ag NPs. The polydisperse character of NPs is suggested by the width of the absorption peak. For various groups, the IR spectroscopy of Ag NPs showed absorption maxima at 3304, 2200, 2066, 1969, 1636, 1261, 1094, and 611. Even though scholars have mentioned the polysaccharide and protein content of the mushroom, they have overlooked its stretching frequencies in the IR spectroscopy. The peak at 3304, on the other hand, has been attributed to (OH) of carboxylic acid, and the peaks at 2200 and 1969 cm⁻¹ have been linked to

unsaturated aldehydes. Unsaturated alkaloids are the cause of the other peaks below 1500 cm⁻¹. Ag NPs appeared to be spherical with an average size between 20 and 30 nm according to the high-resolution transmission electron micrograph and field emission scanning electron. (Sanghi R, Verma P. et al, 2009). Al-Bahrani et al.'s (Al-Bahrani R, Raman J, Lakshmanan H, Hassan AA, Sabaratnam et al, 2017) publication on the biogenic production of Ag NPs from *Pleurotus ostreatus*, a tree oyster mushroom, is recent. 1 mM AgNO₃ and dried aqueous mushroom extract (1-6 mg/mL) were combined and incubated in the dark for 6-40 hours. The creation of Ag NPs was indicated by the hue changing from pale yellow to dark brownish yellow. At 420 nm, a prominent and wide absorption band was visible in the UV-vis spectroscopy. They are polydisperse NP with an average size of 28 nm and a size range of 10-40 nm. Numerous fungi have been employed for the extracellular and intracellular production of Ag NPs, including *Aspergillus flavus*, *Aspergillus fumigates*, *Fusarium oxysporum*, *Fusarium acuminatum*, *Fusarium culmorum*, *Fusarium solani*, *Metarhizium anisopliae*, *Phoma glomerata*, *Phytophthora infestans*, *Trichoderma varied*, and There are many sizes of these NPs. (Vigneshwaran N, Ashtaputre NM, Varadarajan PV, Nachane RP, Paralikar KM, Balasubramanya et al, 2007).

2.5. PLANT SYNTHESIZING Ag NPs:

Plant-related components such leaves, stems, roots, shoots, flowers, barks, seeds, and their metabolites have all been employed effectively to produce NPs (Husen A. et al, 2017). (Beg M, Maji A, Mandal AK, Das S, Aktara MN, Jha PK, Hossain et al, 2017) research on the green production of Ag NPs from *Pongamia pinnata* seed extract is recent. An absorption maximum at 439 nm proved that NPs were formed. The zeta potential of the evenly distributed NPS, which had an average size of 16.4 nm, was equal to or less than 23.7 mV, supporting their stability and dispersion. Ag NPs and human serum albumin did not interact significantly, according to the research. Karatoprak et al. (Karatoprak GS, Aydin G, Altinsoy B, Altinkaynak C, Kos M, Ocsoy et al 2017) have described the green synthesis of Ag NPs from the medicinal plant extract *Pelargonium endlicherianum* in a fairly recent article. Gallic acid, apocyanin, and quercetin from the plant function as reducing Agents to create Ag NPs. Moldovan et al. reported on the Phyto mediated production of spherical Ag NPs from *Sambucus nigra* fruit extract. (Moldovan B, David L, Achim M, Clichici S, Filip GA et al, 2016) They were crystallised, according to an XRD examination. Investigating the in vivo

antioxidant activity on Wistar rats revealed excellent results. It implies that modifying Ag NPs with natural phytochemicals may shield cell proteins from the formation of ROS. Additionally, aqueous leaf extract of *Artocarpus altilis* was used to create Ag NPs. They had a weak antibacterial and antioxidant effect. Based on the occurrence of a sharp peak at 420 nm in the UV-vis region of the spectroscopy, it has been determined that *Thalictrum foliolosum* root extract mediates Ag NPs formation (Hazarika SN, Gupta K, Shamin KNAM et al,2016). The face-centred cubic geometry of the 15-30 nm monodispersed spherical nanoparticle. According to Logaranjan et al., the regulated production of Ag NPs from aloe vera plant extract and their antibacterial effectiveness were observed. (Logaranjan K, Raiza AJ, Gopinath SCB, Chen Y, Pandian K et al,2016).

2.6 APPLICATION OF Ag NPs:

Due to their special properties, Ag NPs are of particular interest in contemporary nanotechnology research. These properties allow for the incorporation of Ag NPs into a wide range of applications, including antiseptics in the medical industry, cosmetics, food packaging, bioengineering, electrochemistry, catalysis, and environmental uses. Noble NPs have differing catalytic activity as compared to their bulk components, and as a result, nano-catalysis, which employs NPs as catalysts in a variety of processes, has recently attracted a lot of attention. For instance, well-known catalysts in the conversion of H_2O_2 to oxygen include gold, Ag, platinum, and metal ions (Merge et al. 2007). The catalytic potential of Ag NPs was found to be greater than that of gold and platinum NPs in the emission system of chemiluminescence from luminol- H_2O_2 (Guo et al. 2008). Moreover, the use of Ag NPs immobilised on silica spheres can improve the catalysis of the reduction of dyes by sodium borohydride ($NaBH_4$). The pace of reaction was nearly motionless in the absence of Ag NPs acting as catalysts, indicating that very little or even no reduction of the dyes had taken place (Guo et al. 2008). Numerous medical applications impregnated with Ag NPs, such as catheters and cardiovascular and bone implants, have been recognised for preventing the formation of biofilm and reducing the risk of pathogenic invasion due to the significant antimicrobial properties of Ag NPs Against a wide range of microorganisms (Tran et al. 2013). Due to its high vulnerability to wear and tear, ultrahigh molecular weight polyethylene has often been utilised extensively as an insert for artificial joint replacement, but its applicability is rather constrained (Morley et al. 2007). However,

the inclusion of Ag NPs considerably reduces the disadvantage of polymer wear and tear. Additionally, it is loaded with polymethyl methacrylate, which is commonly used in bone cements, which are widely employed as synthetic joint replacements due to the strong antibacterial qualities of Ag NPs (Alt et al. 2004). (Xing et al.2010) concluded from their thorough investigation of both osteoblast (bone cells) and fibroblast (skin cells) cultured on such scaffolds that (poly-(3-hydroxybutyrate-co-3-hydroxyvalerate) PHBV nanofiber scaffolds containing Ag NPs have the tendency to aid in bone and skin tissue regeneration. Therefore, by manufacturing the surface of the bone implants devices and scaffolds with Ag NPs, the risk associated with implantation surgery can be reduced (Xing et al. 2010). Additionally, nano-crystalline dressings containing Ag NPs are used to treat wounds or hospital-acquired infections while reducing the inflammatory response (Fong and Wood 2006). For instance, the traditional surgical meshes are utilised for tissue therapy and to bridge serious wounds, but they are extremely susceptible to pathogenic invasions. Thus, the addition of Ag NPs to these meshes improves their efficacy. As opposed to typical fluorescent dyes, Ag NPs plasmonic capabilities make them broadly applicable in bio-imaging for monitoring dynamic events over time without photo-bleaching. In order to help eliminate unwanted or damaged cells, the conjugation of cells to the target cells results in the conversion of light energy to thermal energy, which subsequently causes the target cells to be thermally ablated (Loo et al. 2005). Additionally, Ag NPs plasmonic capabilities can be used for bio-sensing, which effectively detects a variety of proteins that conventional biosensors cannot. Ag NPs are widely used for identifying numerous abnormalities and diseases in the human body system due to their special ability, such as tumour cells or cancer. According to (Morley et al. 2007), the size, shape, and dielectric potential of the surrounding medium all influence the plasmonic characteristics of Ag NPs.

2.7. Ag NPs AND THEIR ANTIBACTERIAL ROLE:

The pharmaceutical firms have spent the past 30 years concentrating on creating new antibiotics that are better able to combat DNA replication, protein synthesis, and the creation of bacterial cell walls. Due to the growth in antibiotic resistance in bacterial infections, there is still a significant fatality rate despite this progress. The worldwide impact of bacterial resistance to conventional antibiotics on healthcare is significant (Surwade.P et al.2017). The use of nanomaterials is expanding and playing an

increasingly significant role in our daily lives. The search is on for low-cost, non-toxic materials that have a variety of uses in the industrial, medicinal, pharmaceutical, and cosmetic fields. Controlling drug-resistant microorganisms and illnesses is a crucial application of such materials (Shim et al, 2018). Ag NPs interact with the bacterial cell membrane, as Agreed upon by scientists, although the main biological target is yet unclear. Antibacterial Ag NPs are effective Against 650 different diseases. Ag NPs coupled with conventional antibiotics have recently been shown to have antibacterial effect, particularly Against multidrug resistant pathogens like *Staphylococcus aureus* and *Escherichia coli*. (Yuwen.R et al,2017). When compared to particles made from other heavy metals like gold, platinum, and zinc, Ag NPs have demonstrated a high antibacterial activity and low cytotoxicity levels. They may bind to cells, impede enzyme activity, cause cell membrane instability, and ultimately result in cell death (Tang. S et al.2018).

CHAPTER 3

AIM AND SCOPE OF THE STUDY

3.1. AIM:

To evaluate biosynthesis of Ag NPs using microorganisms.

2. OBJECTIVES:

- Isolation and identification of bacteria.
- Sub culturing of bacteria.
- Synthesis of Ag NPs by bio reduction.
- characterisation of Ag NPs by UV-Vis spectroscopy, particle size analyser and zeta potential.
- Optimization of Ag NPs.
- Checking antioxidant activity using DPPH assay.

3. SCOPE OF THE STUDY:

Ag NPs are used in the fields of antibacterial Agents, electro electric Sensors, biosensors, health care and agricultural technology. Against both grams positive and gram-negative pathogens, they represent a great bactericidal potential. As Ag NPs are active Against many drugs resistant bacteria and can be used as an easily accessible treatment for several infections, it is appropriate to use them in combination with antibiotics. An Ag NPs is very commonly used to increase solubility in a drug delivery system in a wide range of industrial technologies, biotechnology and Agriculture NPS are investigated in large numbers. It is known that various forms of Ag from laundry, paints, clothes etc. and bio solids reach the sewage and sludge. As a result of the reaction between Ag NPs, Ag⁺ ions and Sulphides produced in wastewater. Nano sized Ag have been reported to form in activated sludge.

CHAPTER 4

4. MATERIALS AND METHOD

1. MATERIAL REQUIRED:

1. Bacterial extract
2. 1mM AgNO₃
3. HCL
4. NaOH
5. Zobel marine Agar
6. Agar Agar
7. Autoclave water
8. Test tubes
9. Water bath
10. Hot air oven
11. Weighing machine
12. Laminar air flow (LAF)
13. Petri plates
14. Stopwatch
15. DPPH
16. Centrifuge
17. Autoclave
18. Ethanol
19. Spirit lamp
20. Cotton
21. Mother culture
22. Inoculation loop

2. METHOD:

ISOLATION AND IDENTIFICATION OF MARINE BACTERIA

1. SAMPLE COLLECTION:

Sea water from Kovalam beach, East coast of Tamil Nādu was collected at a depth of 2- 10 meters. The sample was collected in PET bottles and stored at 4 °C in an ice Bag. the collected sample was processed on that day itself. The collected sample was taken for serial dilution followed by spread plate method.

3. MEDIA PREPARATION:

All the glassware was properly sterilized. 250ml of distilled water was taken in 500ml flask. Carefully 42.9g of Zobell marine Agar was dissolved with the distilled water and 4g of Agar Agar was also added. The conical flask was covered completely and kept in autoclave for 2 hrs at 121°C. After 2 hrs it Was taken from autoclave and poured at 10 petri plates equally. The petri dishes were kept in UV for 15min. The plates were taken for sub culturing.

4. ISOLATION OF BACTERIA:

7 test tubes were taken for serial dilution. 9 ml of distilled water was added in each test tube. one ml of the sea water that is the sample was added in the first test tube to makeup 10^{-1} dilution. By repeatedly emptying and filling the pipette, the dilution is thoroughly blended. A fresh pipette tip is added to the pipette after the old one is removed. The mixture is now withdrawn in 1 ml from the 10^{-1} dilution and poured into the second tube. The overall dilution factor in the second tube is now 10^{-2} . The remaining tube is then subjected to the identical procedure, with 1 ml from the first tube being added to the subsequent 9 ml of diluents. The final dilution of the bacteria/cells will be 10^{-6} (1 in 1,000,000) because six tubes were utilised. The diluted sample was spread on a petri dish with Zobell marine Agar. The culture was left for incubation at 37°C until growth.

5. SUBCUTLURING OF BACTERIA:

Culture sample were taken from isolation plates for subculture. The streaking was done under LAF. It was made sure that the LAF was properly cleansed with ethanol and cotton and the spirit lamp was on before opening the culture. Carefully the inoculation loop was incinerated and from the mother culture small colonies were streaked. Sample cultures were streaked in Zobel marine Agar and incubated at 37°C until growth. The sample was further sub cultured until contamination was undetectable. This sample was considered as potential candidate for synthesizing Ag NPs as it was able to reduce AgNO_3 to Ag NPs. All these strains were periodically sub cultured to maintain the culture for further studies. Sterilization of all glassware's were done before sub culturing.

6. IDENTIFICATION OF BACTERIA BY GRAMSTAINING:

The slides were correctly cleansed and neatly marked to prevent confusion. On the mark slide, a drop of distilled water was added. The microscopic slide was covered with a smear using the inoculation loop. The slide was heated fixed before being subjected to the staining procedure. Applying crystal violet as a main stain. Adding Gram's iodine as a mordant. Rapid decolourization with acetone, ethanol, or a combination of the two. And finally using safranin as a counterstain. With the interval of 1 min before adding each stain. The slide was observed in phase contrast microscope.

7. PREPARATION OF AgNO_3 SOLUTION:

1mM of AgNO_3 solution was prepared. Carefully 0.0017mg of AgNO_3 was weighed under light sensitive zone. The weighed sample was diluted in 100ml of distilled water as measured. The solution was poured into a falcon tube and wrapped with aluminium foil. AgNO_3 get easily reduced by light, so it is important to make sure the solution isn't exposed more to light.

8. SYNTHESIS OF Ag NPs:

The synthesis was based on molarity with the mechanism of bio reduction. Different molarity like 0.1 mM, 0.5 mM, 1 mM, 0.005 mM and 0.001 Mm concentrations were taken by varying sample conc and AgNO_3 conc. The ratio used was 2:1.

TABLE 4.1: BASED ON DIFFERENT MOLARITY:

MOLARITY (m M)	<i>B. cereus</i> (ml)	H₂O(ml)	AgNO₃ (ml)
0.1	0.2	1.8	1
0.5	1	1	1
1	2	0	1
0.01	0.02	2	1
0.05	0.1	1.9	1

The sample was added along with autoclave water and AgNO₃.

4.9: MECHANISM INVOLVED:

Ions that cause NPs production intra or extracellular production of inorganic compounds is common among bacteria. However, with various biological agents, the process for intra and extracellular creation of NPs differs. The intracellular technique makes use of a unique ion transport system within the microbial cell. The cell wall of the microorganisms is crucial in the intracellular creation of NPs. The mechanism entails the electrostatic interaction of the cell wall's negative charge and the metal ions' positive charges. The ions are converted to NPs by the enzymes found inside the cell wall, and these NPs disperse outside the cell wall. The creation of NPs comprises trapping, bio reduction, and capping, according to a sequential procedure for intracellular synthesis of NPs employing bacterial sp. The fungal cell surface interacts electrostatically and retains the metal ions when they come into touch with it. Metal ions are converted to metal NPs by the enzymes found in cell walls. As a result, the bacterial cell and metal clusters interact electrostatically, resulting to the creation of nanoclusters. Later, the tiny nanoclusters get through the bacterial cell wall and are transferred.

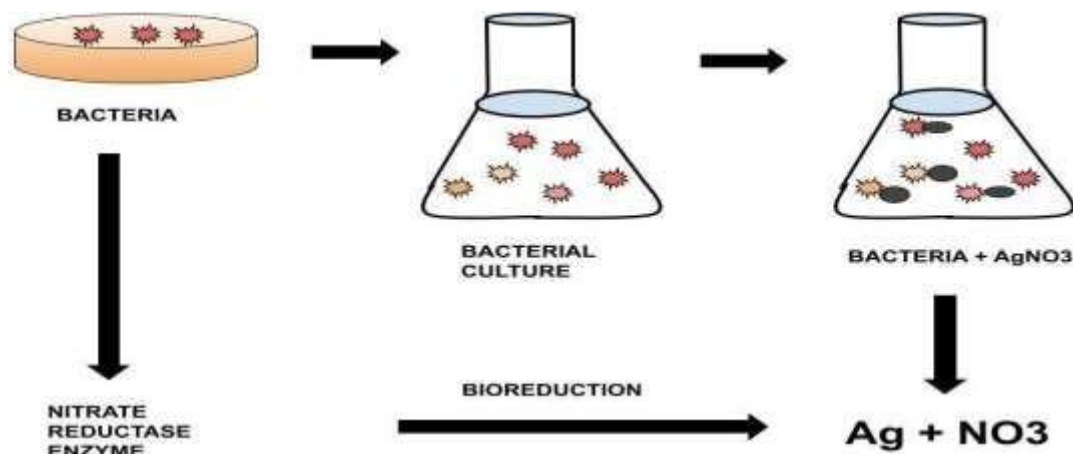


Fig 4.1: VISUAL REPRESENTATION OF MECHANISM INVOLVED:

10. : BULK SYNTHESIS OF AG NPs:

The Ag NPs were bulk synthesised by preparing 50ml of *B. cereus* sample in a conical flask. The sample was kept in a magnetic stirrer to increase the temperature and AgNO₃ solution of 1mM was added to make up the solution to 60ml. it was made sure that conical flask was wrapped around aluminium foil. The colour change was observed to yellowish brown.

11. CENTRIFUGATION AG NPs:

The bulk synthesised sample was transferred to a falcon tube and centrifuged at 6000 rpm at 21°C for 20 min. The colour less supernatant solution was discarded, and the pellet was collected and further centrifuged. The final centrifuged pellet was dried in hot air oven and the sample was given for characterisation.

12. CHARACTERISATION TECHNIQUES:

The bulk synthesised Ag NPs obtained with the 0.05 molarity was given for characterisation. the synthesized sample was observed for its stability before giving it for characterisation. UV-Vis spectroscopy confirms the synthesis of Ag NPs. The particle size analyser is used to determine the size of individual particles as well as the size distribution range for the given sample.

1. UV–VIS SPECTROSCOPY:

It was done at centre for ocean research and development in Sathyabama institute of science and technology. The absorption range was between 200nm – 600nm. This method is most frequently used to monitor the stability and production of metallic NPs in order to characterise them. Surface Plasmon resonance absorption bands are created by the free movement of electrons in these bands. The chemical environment, the dielectric medium, and the particle size all affect how well Ag NPs absorb light. For a number of metals NPs, examination and investigation of the surface Plasmon peak is well known. The chemical environment, the dielectric medium, and the particle size all affect how well Ag NPs absorb light. For numerous metal NPs with sizes ranging from 2-100 nm, the examination and investigation of the surface Plasmon peak is well known. In order to test the stability of biologically created Ag NPs for around a year, UV-Vis spectroscopy was used to identify a surface plasmon resonance peak at the same wavelength.

2. DLS -PARTICLE SIZE ANALYZER AND ZETA POTENTIAL:

Particle size analyses were carried at centre for ocean research in Sathyabama institute of science and technology, on DLS Disc Centrifuge Model No. nanopartica SZ-100V2. Particle characterisation with a single number is a fundamental issue in particle size analysis. A two-dimensional Graph with the particle size on the x-axis and the amount of material on the y-axis is the preferred format for reporting particle size distributions in most particle sizing techniques.

13. ANTIOXIDANT ACTIVITY:

1. 2,2-DIPHENY- 1-PICRYLHYDRAYL (DPPH) ASSAY:

Preparation of DPPH reagent with sterile glassware 1mM of DPPH was added in 100ml of ethanol. the DPPH solution was stored in brown reagent bottle to avoid the exposure of light. the colour of DPPH was purple. The free radical scavenging activity and standard BSA was determined using stable radicle DPPH. Different concentration of Ag NPs of 200µl, 400 µl, 600 µl, 800 µl, and 1000 µl were taken and 2ml of 0.1 mM freshly prepared DPPH solution was added (in ethanol). Then the solution was incubated at room temperature in the dark for 30 min. The colour changed proved the

antioxidant activity of the Ag NPs. The absorbance was recorded at 517nm using UV Vis spectroscopy.

14. OPTIMIZATION OF Ag NPs:

The Ag NPs were synthesised by changing different parameters like temperature, pH, and reaction time. It was also studied that, how the influence of pH, temp, and time effect the synthesis of stable Ag NPs. The experiment led to the synthesis of stable Ag NPs. The NPs did not settle, nor did they form any Aggregate.

1. BASED ON pH:

The influence of pH on the formation of Ag NPs was examined by keeping the pH of the reaction medium (which is obtained by 0.05 concentration of bacteria [1ml] and 1mM of AgNO₃ [1ml]) at 3, 6, 7, 9, 12. The pH was altered by adding HCL and NaOH. The color change was noted.

TABLE 5.1.1: SYNTHESIS OF AgNO₃ BY VARYING pH:

MOLARITY	pH	OBSERVATION
0.05	3	No change
0.05	6	No change
0.05	7	No change
0.05	9	Yellow colour
0.05	12	Yellow colour

4.14.2. BASED ON REACTION TIME:

To optimize the reaction time, the biosynthesis of Ag NPs was monitored periodically in a UV-vis spectroscopy. The reaction mixture comprising 1ml of 1mM AgNO₃ and 3ml of *B. Cereus* bacteria (0.05 conc.) at room temperature as a function of reaction time from 0 min to 2 hrs. 7 test tubes were taken in total and in the appropriate time the sample was added in the microliter plate for the reading.

TABEL: 5.1.2. SYTHESIS OF AgNO_3 , WITH INFLUENCE OF TIME:

MOLARITY	TIME	OBSERVATION
0.05	0 TH SEC	Yellow colour
0.05	5 th min	Yellow colour
0.05	10 th min	Yellow colour
0.05	20 th min	Yellow colour
0.05	40 th min	Yellow colour
0.05	1hr	Yellow colour
0.05	2hr	Yellow colour

4.14.3. BASED ON TEMPERATURE:

To analyze the influence of temperature on the biosynthesis of AgNO_3 the reaction mixtures were maintained at five different temperatures namely 12, 20, 37, 70 and 100° C. every 5 minute the test tubes kept at different temperatures were observed for color change. The temp. was measured using a thermometer before adding AgNO_3 solution.

TABLE 5.1.3: SYNTHESIS OF AGNO_3 , WITH THE INFLUENCE OF TEMPERATURE:

MOLARITY	TEMPERATURE	OBSERVATION
0.05	11°C	No change
0.05	20°C	No change
0.05	37°C	Brown colour
0.05	70°C	Brown colour
0.05	100°C	Light change to brown

CHAPTER 5

RESULTS AND DISCUSSIONS:

5.1 ISOLATION AND IDENTIFICATION OF BACTERIA:

By using a spread plate approach followed by repeated dilution, the bacteria were successfully recovered from sea water. Further, the bacteria were sub cultured using the quadrant streaking technique of sub culturing (Fig 5.1). After 24 hours, the bacteria's growth stopped. Gram staining was used to identify the subcultures microorganisms. *B. cereus* was found to be a gram-positive, spore- forming bacteria.



FIG 5.1: ISOLATION AND IDENTIFICATION OF BACTERIA BY GRAM STAINING.

5.2: SYNTHESIS OF Ag NPS AND CHARACTERISATION:

The initial sign of nanoparticle creation is a change in colour from colourless solution to yellowish brown, which is further supported by UV-Vis spectroscopy. The colour of the treated solution changed from colourless to yellowish brown, indicating that the concentration of aqueous AgNO_3 ions was decreased after exposure to *B. cereus* biomass. On the concentration of 0.05mM the colour was stable and constant. This particular concentration was bulk synthesized, and the result was same as observed earlier. The stimulation of surface plasmon vibrations in the Ag metal NPs causes the

distinctive yellowish-brown colour to appear. Thus, UV-Vis spectroscopy makes it simple to monitor the biomass reduction of AgNO_3 ions.



FIG 5.2: SYNTHESIS OF Ag NPS BASED ON DIFFERENT MOLARITY



FIG 5.3: BULK SYNTHESIS OF Ag NPs WITH 0.05 MOLARITY, FORMATION OF Ag NPs BY COLOUR CHANGE.

5.3 UV-Vis SPECTROSCOPY:

By taking a sample of the aqueous component and measuring the UV-Vis spectroscopy of the solution, as shown in (Fig.5.4) UV-vis spectroscopy was used to characterise the reduction of the Ag ions by the supernatant of the test bacteria in the

solution and the formation of Ag NPs. These samples were obtained using an UV-Vis spectroscopy with a 1nm resolution. The surface plasmon band in the Ag NPs solution stays close to 440 nm throughout the reaction period, indicating that there is no indication of Aggregation and that the particles are disseminated throughout the aqueous solution. The peak at 440nm indicated the presence of Ag NPs.

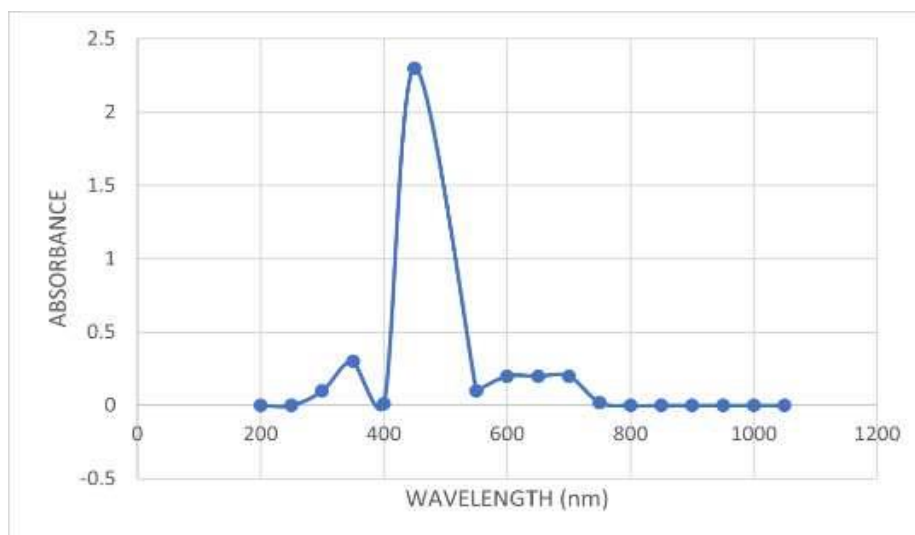


FIG 5.4: UV-VIS SPECTROSCOPY OF Ag NPs.

5.4: PARTICLE SIZE AND ZETA POTENTIAL ANALYSIS:

The DLS size distribution Image of Ag NPs produced during biosynthesis is displayed in (Fig. 5.5). Ag NPs size distribution is found to range from 10 to 400 nm. The Z-average (r. nm), according to DLS data, was 387.8 nm. The synthesised Ag NPs had an average size of 0.769 Pdl value. The solitary peak that was found demonstrated that the synthesised Ag NPs were of high quality. The biosynthesized Ag NPs ' zeta potential was discovered to be a strong peak at 32.8 mV (Fig. 5.6). It is hypothesised that the NPS' surfaces are negatively charged and diffused throughout the liquid. The fact that the value is negative demonstrates how strongly attracted and repulsed the particles are to one another.

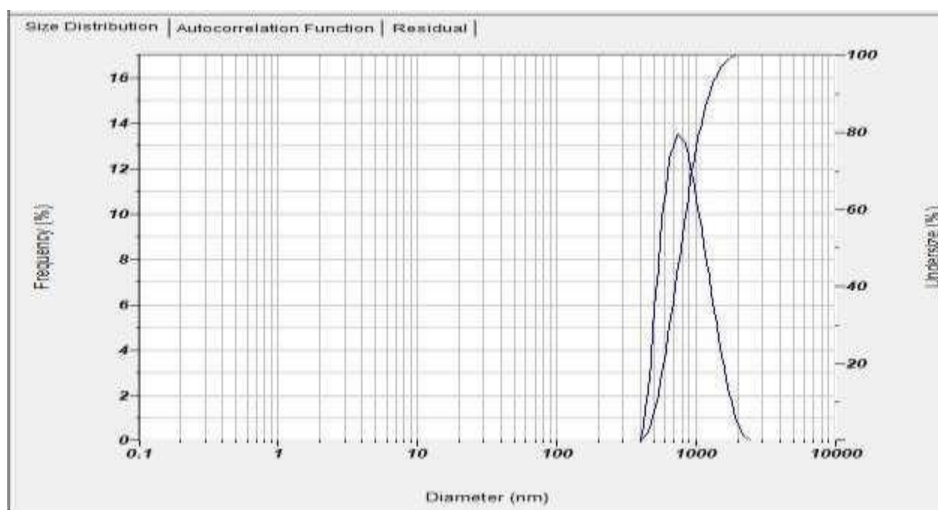


FIG 5.5: PARTICLE SIZE ANALYZER

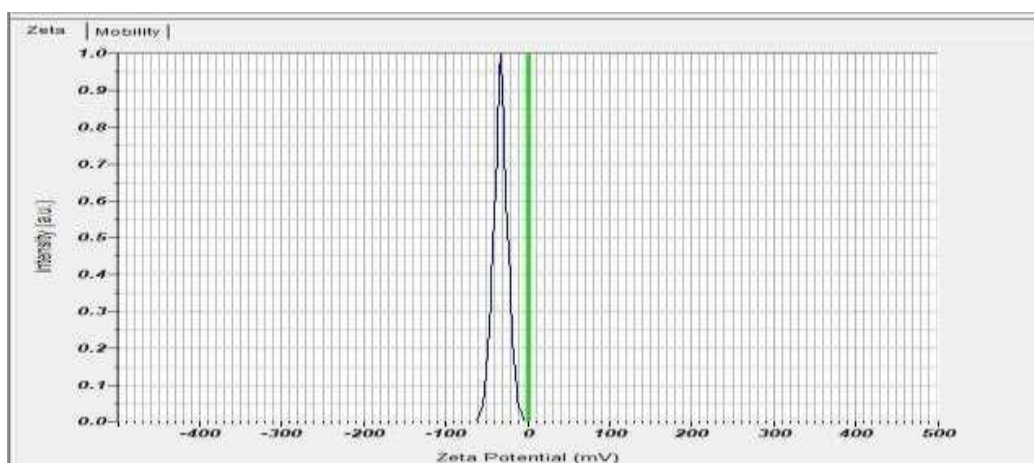


FIG 5.6: ZETA POTENTIAL ANALYZER

5. : OPTIMIZATION OF Ag NPS:

1. BASED ON pH:

Ag NPs were made using optimised parameters. After 30 minutes, the solution changed colour from clear to brownish yellow after the addition of aqueous AgNO_3 . Ag NPs are formed when the colour of brown changes. There is no colour change at pH 3, which shows that Ag NPs do not grow at an acidic pH. At pH 9, colour changes occurred quickly, and the peak of Ag 's absorbance changed to 500 nm as a result of Ag NP Agglomeration. Immediately after adding the AgNO_3 to the reaction mixture, Aggregation was seen at pH 9 in the sample. pH 6 was almost neutral; no

colour change occurred. The typical UV-vis spectroscopy absorption peak at 420 nm verified the creation of Ag NPs, which are Ag NPs.



FIG 5.7: SYNTHESIS OF Ag NPs BASED ON DIFFERENT pH.

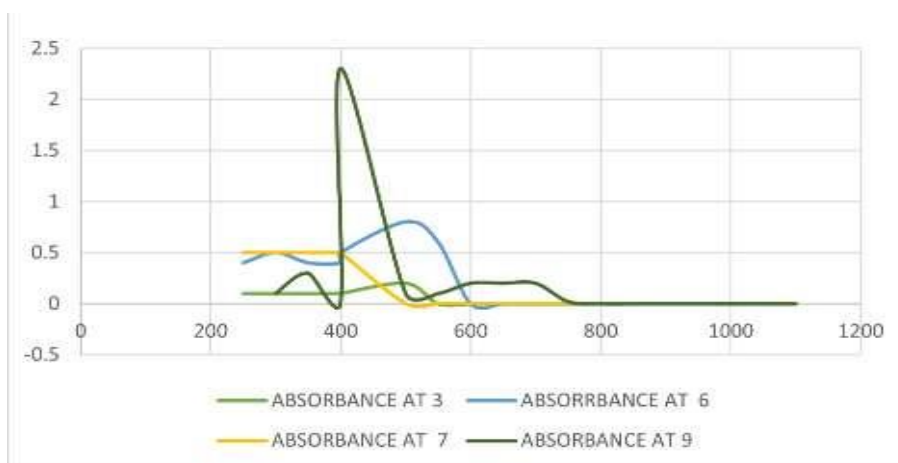


FIG.5.8: UV-VIS SPECTROSCOPY PEAKS OF DIFFERENT pH

5.5.2 : BASED ON REACTION TIME:

The synthesis of Ag NPs was done periodically, and colour changed was observed every minute. The time intervals of 0, 5, 10, 20, 40, 1 hr and 2 hrs were taken. The UV-Vis spectroscopy peak was formed at 412 nm in the time 20th min. the colour was constant and stable. No Aggregates were formed.



FIG 5.9. SYNTHESIS OF Ag NPs BASED ON REACTION TIME.

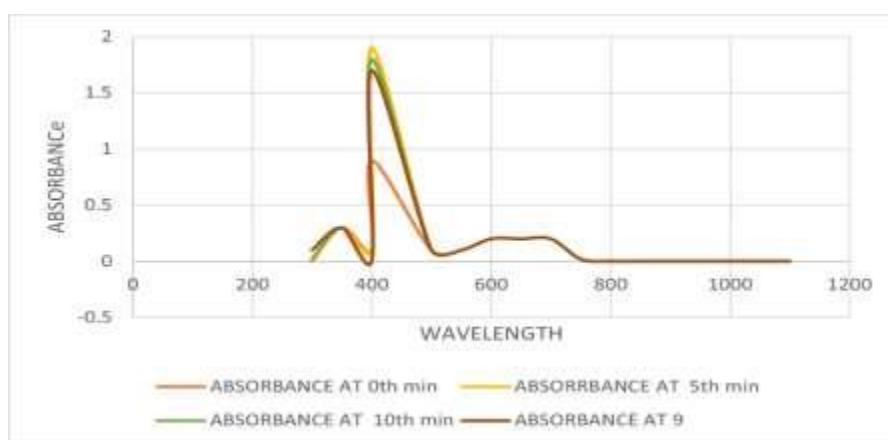
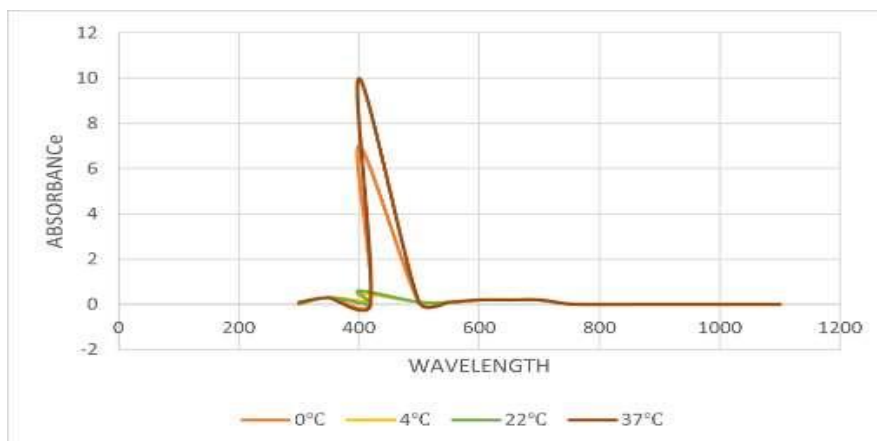


FIG 5.10: UV-VIS SPECTROSCOPY FOR REACTION TIME

5.5.3 BASED ON TEMPERATURE:

At various temperatures, Ag NPs synthesis was carried out. However, the colour did not change at 0°C or 4°C, indicating that these temperatures were not ideal to produce Ag NPs. The colour changed and Ag NPs developed at normal temperature. There was a very slight colour shift at 22°C. The solution evaporated at 100°C. As a result, the highest peak was seen in the synthesised Ag NPs at room temperature.



A

B

FIG 5.11(A&B): Ag NPS FORMATION AT 37°C UV-VIS SPECTROSCOPIES BASED ON VARIOUS TEMPERATURE.

5.6. ANTIOXIDANT ACTIVITY:

Using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a free radical scavenger, the antioxidant activity of Ag NPs was evaluated. In this test, removing the H-atom from the antioxidant molecule causes DPPH to turn pale yellow instead of its original violet colour. The assay reaction mixture consisted of 2 ml (0.1 mM) of DPPH and 1 mL of Ag NPs at varied concentrations (10, 20, 30, 40, and 50 mg/mL) and was incubated at 37°C for 0.5 hours with vigorous shaking. The mixture's absorbance was gauged at 517 nm, and BSA served as the positive control.



FIG 5.12: COLOUR CHANGE FROM VIOLET TO ORANGE INDICATING ANTIOXIDANT ACTIVITY.

CHAPTER 6

SUMMARY AND CONCLUSIONS

6.1. SUMMARY:

Due to the fundamental drawbacks of Physical and chemical synthesis methods, biological routes to synthesis using microorganisms are considered a safe alternative. These microbes exhibit highly structured physical and biosynthetic activity, allowing us to obtain NPs with controlled shape and size. The demand for benign synthesis of Ag NPs has led to this new study in which Endophytic bacteria were used for their synthesis. The potential of endophytes has been previously demonstrated in that it plays a role in controlling plant pathogens, insects and nematodes, accelerating seedling emergence and plant growth, and promoting plant establishment under adverse conditions. It has been. Bacterial endophytes have been shown to prevent disease development through Endophyte-mediated de novo synthesis of novel compounds and antifungal metabolites. Their versatile abilities inspired the idea of using them in the synthesis of NPs in current research. The Usage of Ag NPs, which are demonstrating to be potent antibacterial Agents, has overcome the restricted effectiveness of Ag and its derivatives Against microbes due to their interfering effects of salts. Additionally, antibacterial activity Against all of the test species used was discovered in the biosynthesized Ag NPs in the current investigation. This opens a new line of inquiry where endophytic bacteria can also be utilised in the environmentally friendly creation of NPs.

6.2. CONCLUSION:

Biosynthesis of Ag NPs using marine bacteria, *B. cereus* was resulted successfully. firstly, collecting the bacteria from the sea, then using for synthesis Ag NPs. followed by characterisation with, particle analyzer and UV-Vis Spectroscopy. Particle size analyser identifying the particle size, particle shape, and surface properties. UV – Spectroscopy identifies the absorbance of the material is studied as a function of wavelength. Numerous pathogenic bacteria, such as *E. coli*, *B. licheniformis*, and *R. solanacearum*, were able to be inhibited by the produced Ag NPs. Due to its numerous uses as antibacterial and antitumor agents as well as in food packaging, agriculture, and the medical field, Ag NPs play a key role in the management of health. Furthermore, it is common knowledge that the majority of empirical antibiotic use results in resistance and ineffectiveness. Therefore, bacteria that form biofilms pose a significant threat. Alternative therapy approaches are receiving more attention globally as a solution to the antibiotic resistance issue. Among these alternate tactics is the possible application of Ag NPs as antibiofilm agents, as well as surface coating or impregnation of nanomaterials. Ag NPs are also the most researched and used Ag NPs in the treatment of many ailments, including cancer, wound healing, dental implants, and other therapies including regulating biological activity. The use of these new particles in medicine will create a standard platform for the prevention and treatment of multidrug resistance and biofilm infections with improved knowledge and technology.

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