Biological Synthesis and Characterization of Silver Nanoparticles using *Bacillus thuringiensis*

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By

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CERTIFICATE

This is to certify that the thesis entitled "**Biological synthesis and characterization of silver nanoparticles using *Bacillus thuringiensis***" which is being submitted by **Miss. LILYPRAVA DASH** Roll No. **411LS2044,** for the award of the degree of Master of Sciencefrom National Institute of Technology, Rourkela, is a record of bonafied research work, carried out by her under my supervision. The results embodied in this thesis are new and have not been submitted to any other university or institution for the award of any degree or diploma.

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DECLARATION

I hereby declare that the thesis entitled "**Biological synthesis and characterization of silver nanoparticles using *Bacillus thuringiensis***", that I submitted to the Department of Life Science, National Institute of Technology, Rourkela for the partial fulfillment of the Master Degree in Life Science is a record of bonafied and original research work carried out by me under the guidance and supervision of Dr. Suman Jha , Assistant Professor, Department of Life Science, National Institute of Technology, Rourkela. To the best of my knowledge no part of this thesis has been submitted to any other university or institution for the award of any degree or diploma.

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ABSTRACT

The present work investigates the synthesis of silver nanoparticles (Ag NPs) by biological method using *Bacillus thuringiensis*. The MIC test was performed to test the inhibitory concentration of $AgNO_3$ against *B. thuringiensis* and *B. subtilis*. Silver nanoparticles were successfully synthesized from *B. thuringiensis* by green synthesis method. The detailed characterization of the Ag NPs was carried out using UV-visible spectroscopy, Scanning Electron Microscopy (SEM), X Ray Diffraction (XRD), and FTIR. From the UV-visible spectroscopy, the absorption peak was found at 420 nm. From the SEM images, it is confirmed that the sample contains spherical silver nanoparticles. From XRD analysis, it was confirmed that the silver nanoparticles are crystalline in nature, which was confirmed by the FT-IR peak at 518 cm^{-1} corresponding to the Ag vibration present in crystalline structure. Finally, the antibacterial activity of silver nanoaprticles was determined by spread plate method, and found that silver nanoparticles have significant antibacterial activity against *S. aureus and B. subtilis than E. coli.*

Key words: Nanotechnology, Silver nanoparticles, and Antibacterial activity.

INTRODUCTION

1.1. Nanoparticles: Building Blocks for Nanotechnology *(R. Nagarajan et al)*

Nanoparticles serve as the fundamental building blocks for various nanotechnology applications. Nanotechnology, and alongside nanostructured materials, play an ever increasing role in science, research and development as well as also in every day's life, as more and more products based on nanostructured materials are introduced to the market. Nanotechnology deals with materials with dimensions of nanometers, i.e. nanostructured materials.The sizes of nanometer dimensionsdetermined the physical and chemical properties of materials undergo extreme changes, which open up a wide range of future, but partly already realized applications. There are two more forces driving towards nanotechnology, mostly biomolecules and other bioentities are of nanometer size; thus the nanoscale provides the best opportunity to study such bio-entities and their interactions with other materials. Another impetus is semiconductor industry which, by its ever-lasting demand for miniaturization, has been driven deeply into the nano-realm. Nanotechnology deals with materials have dimensions in the nanometer range (<100 nm). They may consist of single, isolated nanoparticles, of assemblies of such nanoparticles, of thin films which are two-dimensional nanostructures, of composites in which the constituents are of nanosize dimensions (nanocomposites). Nanosized structures created by lithography and etching as for example semiconductor devices, or of structures created by self-organization ofindividualnanostructures *(W. Kulisch et al). Nanoparticles are seen as solution to many technology and environmental challenges. By green synthesis methods of NPs reduced hazardous to the global efforts (Dahl et al,). Implantation of these sustainable processes should adopt the fundamental principles of green chemistry (Alivistoes et al). These principles emphasize on maximizing the efficiency of chemical processes without compromising safety* *concern of the products.*The physical, chemical and biological properties of the nanoparticle change in fundamentally from the properties of both individual atoms/molecules and of the corresponding bulk material.Diverse chemical natureof nanoparticles, including: metals, metal oxides, non-oxide ceramics, carbon and biomolecules. Nanoparticles have several different morphology forms such as spheres, cylinders, platelets, tubes, etc. Consequently, they are designed with surface modifications tailored to meet the needs of specific applications they are going to be used for. The various diversity of the nanoparticles (Figure 1) arising from their wide chemical nature, shapes and morphologies, the medium in which the particles are present, the state of dispersion of the particles and most importantly, the several possible surface modifications the nanoparticles can be subjected to make this an important active field of science.

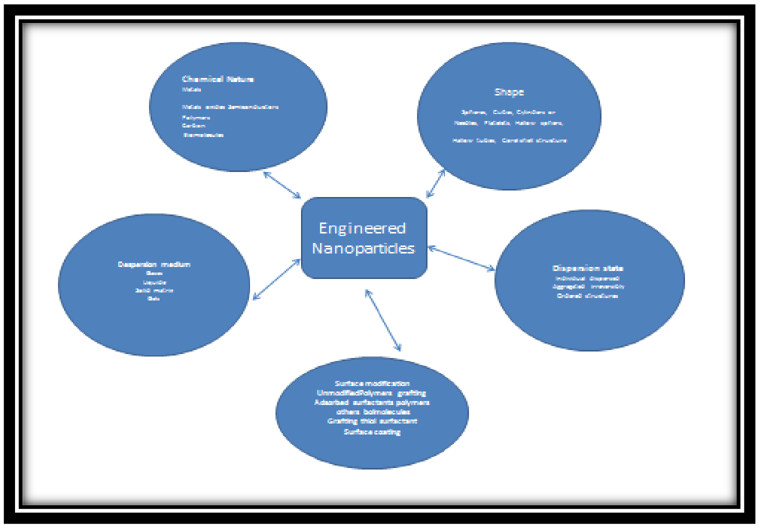


Fig1: V*arious features contributing to the diversity of engineered nanoparticles. The same chemical can generate a wide variety of nanoparticles.*

1.2. Silver Nanoparticles

Silver nanoparticles are one of the promising products in the nanotechnology industry. The development of consistent processes for the synthesis of silver nanoparticles is an important aspect of current nanotechnology research. Silver nanoparticles can be synthesized by several physical, chemical and biological methods. One of such promising process is green synthesis. However for the past few years, various rapid chemical methods have been replaced by green synthesis because of avoiding toxicity of the process and increased quality. Silver nanoparticles have unique optical, electrical, and thermal properties and are incorporated into products that range from photovoltaic to biological and chemical sensors, including pastes, conductive inks and fillers which utilize silver nanoparticles for their high electrical conductivity, stabilization and low sintering temperatures. Due to optical properties of nanoparticles, these are mainly used in molecular diagnostics and photonic devices. An increasingly application is the use of silver nanoparticles for antimicrobial coatings, and many textiles, wound dressing, and biomedical devices contain silver nanoparticles that continuously release a low level of silver ions to provide protection against bacteria.

1.3. Applications of Silver nanoparticles

1) It is used for purification and quality management of air, imaging, and drug delivery system.

2) Biologically synthesized silver nanoparticles have many applications like coatings for solar energy absorption and intercalation material for electrical batteries, as optical receptors, as catalysts in chemical reactions, for biolabelling, and as antimicrobials due to the antibacterial activity.

3) Silver nanoparticles are cytotoxic they have tremendous applications in the field of high sensitivity bimolecular detection and diagnostics, antimicrobials and therapeutics, catalysis and micro-electronics.

4) Potential application of silver nanoparticles like diagnostic biomedical optical imaging, biological implants (like heart valves) and medical application like wound dressings, contraceptive devices, surgical instruments and bone prostheses.

5) Many major consumer goods manufacturers the household products byutilizing the antibacterial properties of silver nanoparticles. These products include nanosilver lined refrigerators, air conditioners and washing machines.

1.4. Silver nanoparticles as an antimicrobial agent

Ag NP highly antimicrobial to several species of bacteria, including the common kitchenmicrobe, *E. coli*. According to the mechanism reported, silver nanoparticles interact with outer membrane ofbacteria, and arrest the respiration and some other metabolic pathway that leads to the death of the bacteria. New technology advances in reducing silver compound chemically to nanoscale sized particles have enabled the integration of this valuableantimicrobial into a larger number of materials—including plastics, coatings, and foams as well as natural and synthetic fibers. Nano-sized silver have already provides a more durable antimicrobial protection, often for the life of the product. Current research in inorganic nanomaterials having good antimicrobial properties has opened anew era in pharmaceutical and medical industries. Silver is the metal of choice as they hold the promise to kill microbes effectively. Silver nanoparticles have been recently known to be a promising antimicrobial agent that acts on a broad range of target sites both extracellularly as well as intracellarly. Silver nanoparticles shows very strong bactericidal activity against gram positive as well as gram negative bacteria including multi-resistant strains (Shrivastava et al., 2007), and also it was found to be in few studies (Zeng et al.,2007; Roe et al., 2008). Hence there is a huge scientific progress in the study of biological application of ZnO and Ag and other metal NPs. The antibacterial effects of Ag salts have been noticedsince antiquity, and Ag is currently used to controlbacterial growth in a variety of applications, including dentalwork, catheters, and burn wounds. In fact, it is wellknown that Ag ions and Ag-based compounds are highlytoxic to microorganisms, showing strong biocidal effects. There is growing interest in understanding the relationship between the physical and chemical properties of nanomaterials and their potential risk to the environment and human health. The availability of panels of nanoparticles where the size, shape, and surface of the nanoparticles are precisely controlled allows for the better correlation of nanoparticle properties to their toxicological effects. Sets of monodisperse and unaggregated, nanoparticles with precisely defined physical and chemical characteristics provide researchers with materials that can be used to understand how nanoparticles interact with biological systems and the environment.

3. Objective

- .Minimum Inhibitory Concentration value for AgNO3 against B. thuringiensisang B. subtilis
- Synthesis of silver nanoparticles using B. Thuringiensis
- Characterization of nanoparticles using different techniques
- Antibacterial activity of silver nanoparticles

3. Review of Literature

3.1. History of Nanoparticles

The development of the concepts and experimental work is the broad category of nanotechnology. Recently nanotechnology is a development in scientific research, the development of its central concepts happened over a longer period of time. Since, people have been preparing the glass windows with tiny colored metal particles especially silver which provide glassy yellow color (Solomon *et al*., 2007).

3.2 Concept of Nanotechnology

The history of nanomaterials is quite long and major developments within nanoscience have taken place during the last two decades. The idea of Nanotechnology was first highlighted by Noble laureate Richard Feynman, in his famous lecture at the California Institute of Technology, 29th December, 1959. In one of his articles published in 1960 titled, "There is plenty of room at the bottom" discussed the idea of nanomaterials. He pointed out that if a bit of information required only 100 atoms, then all the books ever written could be stored in a cube with sides 0.02 inch long. Norio Taniguchi first defined the term Nanotechnology, in 1970. Nanoparticles are being used in several fields including electrical, biological textile and chemistry. Depending up onshape and size of colloidal metal particles play crucial role in different application including preparation of magnetic, electronic devices wound healing, antimicrobial gene expression and in the preparation of bio composites and noble metal colloids have the optical, catalytical electromagnetic properties.

3.3. Classification of nanoparticles

Nanoparticles fall into two categories: mainly organic and inorganic nanoparticles. Organic nanoparticles may include carbon nanoparticles (fullerenes) in other hand, inorganic nanoparticles may include magnetic nanoparticles, noble metal nanoparticles (like gold and silver) and semiconductor nanoparticles (like titanium dioxide and zinc oxide).There is a growing interest in inorganic nanoparticles as they provide superior material properties with functional versatility, have been examined as potential tools for medical imaging as well as for treating diseases due to their size features and advantages over available in chemical imaging drugs agents and drugs. When mesoporous silica combined with molecular machines prove to be excellent imaging and drug releasing systems. Gold nanoparticles have been used extensively in imaging, as drug carriers and in thermo therapy of biological targets (Cheon & Horace, 2009). Inorganic nanoparticles (metallic and semiconductor nanoparticles) exhibit intrinsic optical properties which may enhance the transparency of polymer- particle composites. For that reasons, inorganic nanoparticles have found special interest in studies devoted to optical properties in composites. Size dependent color of gold nanoparticles has been used to color glass for centuries (Caseri, 2009).

3.4. Application of Nanoparticles

Once materials are prepared in the form of very small particles, for that they change significantly their physical and chemical properties. Nano-dimension, percentage of surface molecule compare to bulk molecule is high and this enhances the activity of the particle in nano dimension and the normal properties of the particle like heat treatment, mass transfer, catalytic activity, etc are all increases. But as compare to non-metal nanoparticles, metal nanoparticles have more industrial application. Many new developments are offer by nanoparticles in the field of biosensors, biomedicine and bio nanotechnology-specifically in the areas:

- Drug delivery
- As medical diagnostic tools,

- As a cancer treatment agent (Gold nanoparticles).

Nanoparticles and nanostructure are become developing in human medical application, including imaging or the delivery of therapeutic drugs to cell, tissues and organs. Many drug loaded nanoparticles are interacts with organ and tissues and are taken up by cells. Several studies have shown that the tissue, cell and even cell organelle distribution (Alexiou et al., 2000; Savic et al., 2003) of drugs may be controlled and improved by their entrapment in colloidal nanomaterials, like micellar structure, such as nanocontainer. Magnetic nanoparticles have wide range of applications, such as the immobolization of the proteins and enzymes, bioseparation, immunoassays, drug delivery, and biosensors (Chen.et al., 2002). Nanoparticles of ferromagnetic materials are of important, of their reduced sizes that can support only single magnetic domains.Due to their potential technological application as recording media in synthesis of arrays of 4 nm diameter FePt nanoparticles with an extremely narrow size distribution has promoted a significant research effort (Varlan et al., 1996).

3.5. Properties of Nanoparticles

The presence of a high fraction of atoms/molecules constituting the nanoparticle on the particle surface rather than in the particle interior and the immense surface area available per unit volume of the material is the most significant consequences for the nanoparticles. Both of these properties increase in magnitude with a decreasing particle size. As a result of the unique physical, chemical and biological properties of nanoparticles originate from these two features. Quantum effects of some nanoscale materials are exhibited allowing for a number of interesting applications. The large specific surface area of nanoparticles including: the origin of a number of their unique applications. Catalysis is enhanced by high surface area per unit volume and the homogenous distribution of nanoparticles. However, high surface areas give strong interactions between the nanoparticles and the solid matrix in which they may be incorporated. The platelet morphology and large specific surface areas of silicate nanoparticles particles enhance the barrier properties of polymer membranes by vastly increasing the pathway for molecular transport of permeating substances.

3.6. Physical and *Chemical Properties of nanoparticles*

The principal parameters of nanoparticles are their shape including aspect ratios where appropriate), size, and the morphological sub-structure of the substance. Indirectly chemical agents can stabilize against coagulation or aggregation by conserving particle charge and by modifying the outmost layer of the particle. Depending on the growth characteristics and the lifetime of a nanoparticle very complex compositions will be possibly with complex mixtures of adsorbate. At the nanoparticle - liquid interface, polyelectrolytes have been utilized to modify surface properties and the interactions between particles and their environment. They have been used in a wide range of technologies, including adhesion, stabilization, and controlled flocculation of colloidal dispersions (Liufu et al 2004).

3.7. Nanoparticle - Nanoparticle Interaction

At the nanoscale level, particle-particle interactions are either dominated by weak Van der Waals forces, stronger polar and electrostatic interactions or covalent interactions. In suspended in air, nanoparticles charges can be accumulated by physical processes such as glow discharge or photoemission. In case of liquid, particle charge can be stabilized by electrochemical processes at surfaces. The nanoparticle - nanoparticle interaction forces and nanoparticle – fluid interactions are importance to describe physical and chemical processes, and the temporal evolution of free nanoparticles.The interaction forces, either attractive or repulsive, that determine the fate of individual and collective nanoparticles.

3.8. Commercial application of Nanoparticles

Existing and potential applications involving nanoparticles are almost endless.The amount of nanoparticles usage in different applications may vary significantly. The Project on Emerging Nanotechnologieslists more than 470 products in May 2007, that are verified to include some form of nanotechnology, the number being double that was reported the same time in 2006 (Perez, J et al, 2005). The positive view with which the nanoproducts are being greeted is reflected in the explicit choice of the term 'nano' in the names of many commercial products, for example, Nano & UV Artificial Teeth Cleaner, Nano Air Filter, Nano Anti-Aging Cream, Nano B-12 Vitamin Spray, Nano Bag, Nano Cold Catalyst Air Purifier, Nano Pacifier, Nano Trousers, Nanoceuticals Artichoke Nanoclusters, Nanodesu X Bowling Ball.

Table 1: Present and Potential Applications of Nanoparticles

Biomedical	○ Antibacterial creams and powders (Ag) ○ Biocompatible coatings for implants ○ Biolabeling and detection (Au, Ag, Quantum dots) ○ Cancer diagnostics and targeted drug delivery (magnetic nanoparticles) ○ Cell, receptor, antigen, enzyme imaging (Quantum dots) ○ Fungicides (ZnO, Cu20) ○ Gene delivery (CNT)
Consumer Goods and Personal Care Products	○ Anti-bleaching, scratch resistance additives in paints ○ Anti-scratch coated tiles (alumina) ○ Barrier packaging (silicates)

	o Glass coatings for anti-glare, anti-misting mirrors (Ti02) o Skin creams with antioxidant vitamins (nanocapsules)
Electronics and Computers	o Chemical mechanical planarization (alumina, silica, ceria) o Coatings and joining materials for optical fibers (Si) o Conductive coatings/fabrics (rare-earth-doped ceramics) o Display technologies (conducting oxides) o Electronic circuits (Cu, Al)
Engineering Materials	o Cutting tool bits (A1203, Zr02,WC, TaC) o Thermal spray coating techniques (Ti02 , TiC-Co) o Flame retardant polymer formulations (nanoclay) o Lubricants and sealants/hydraulic additives (Cu MoS)
Environmental	o Self-cleaning glass (Ti02 based coatings) o Soil remediation (Fe) o Water treatment (photo-catalyst treatments, Ti02)
Food	o Flavors and colors in food and beverages (nanocapsules) o Food packaging materials (, Si02, Ti02, Ag)
Power and Energy	o Dye-sensitized solar cells (Ti02 , ZnO, Au) o Environmental catalysts (Ti02 , Ce02 as diesel additive)
Transportation	o Battery technology o High strength, light weight composites for o Increasing fuel o Efficiency

	○ High temperature sensors ○ Improved displays ○ Thermal barrier and wear resistant coatings ○ Wear-resistant tires

3.9.Silver Nanoparticles

The optical properties of silver nanoparticles were used by glass founders as far back as in the time of the Roman Empire. That evidenced, so-called Lycurgus cup (4th century AD) now exposed in the British Museum. A detailed study of the composition of its bronze-mounted insets of stained glass, carried out in the late 20th century, revealed the presence of metal nanoparticles (with the average diameter of 40 nm) that consists of silver (70 %) and gold (30 %) alloy (Barber, 1990). It explained the remarkable feature in bowl to change its color from red in transmitted light to grayish green in reflected light. Before the 1980s, the scientific and practical interest in silver nanoparticles was exclusively caused by the possibility of their use as highly dispersed supports for enhancing the signals from organic molecules in the Raman spectroscopy (Creighton, et al., 1979; Lee et al., 1982).Fundamental studies carried out in the last three decade shows that silver nanoparticles exhibit a rare combination of valuable propertiesincluding, unique optical properties associated with the surface Plasmon resonance (SPR), well-developed surfaces, catalytic activity, high electrical double layer capacitance, etc.(Henglein, 1989). For that reason silver nanoparticles serve as a material in the development of new-generation electronic, optical and sensor devices. In the past 20 years, the trend miniaturization and the necessity of modernization of technological processes led to the substantial increase in the number of scientific publication devoted to the synthesis and properties of silver nanoparticles.

Silver is used as a catalyst for the oxidation of methanol to formaldehyde and ethylene to ethylene oxide (Nagy et al., 1999). This band is attributed to collective excitation of the electron gas in the particles, with a periodic change in electron density at the surface (Henglein, 1989; Ershov et al., 1993). Some studies showed that use of a strong reductant such as borohydride, resulted insmall particles that were somewhat monodisperse, but the generation of larger particles was difficult to control (Creighton et al., 1994; Schneider et al., 1979). Use of a weaker reductant such as citrate, resulted in a slower reduction rate, but the size distribution was far from narrow (Shirtcliffe et al., 1999; Emory et al., 1997). Synthesis of Ag NPs is based on a twosteps reduction process (Schneider et al., 1979). In this technique a strong reducing agent is used to produce small Ag particles, which are enlarged in a secondary step by further reduction with a weaker reducing agent (Lee et al., 1982). Different studies reported the enlargement of particles in the secondary step from about 20–45 nm to 120–170 nm (Schneider et al., 1994; Schirtcliffe et al., 1999; Rivas et al., 2001).

3.10. Synthesis of Ag nanoparticles

Material Scientists researcher is conducting to develop novel materials, with better properties, functionality and lower cost than the existing ones. Different physical, chemical and biological synthesis methods have been developed to enhance the performance of nanoparticles displaying improved properties with the aim to have a better control over the particle size, distribution and morphology (Granqvist et al., 1976; Shibata et al., 1998; Shankar, et al., 2003). Synthesis of nanoparticles to have a better control over particles size, distribution,morphology, purity, quantity and quality, by employing as environment friendly economical processes has always been a challenge for the researchers (Hahn, 1997).

3.11. Synthesis of Ag Nanoparticles by biochemically

Some chemical approaches are the most popular methods for the preparation of nanoparticles. However, some chemical methods cannot avoid the use of toxic chemicals in the synthesis procedure.The noble metal nanoparticles such as gold, silver and platinum nanoparticles are widely applied to human contacting areas, there is a growing need to develop environmentally friendly processes of nanoparticles synthesis that do not use toxic chemicals. Some biological methods of nanoparticles synthesis using microorganism (Nair et al., 2002), enzyme (Willner et al., 2006), and plant or plant extract (Shankar et al., 2004) have been suggested as possible ecofriendly alternatives to chemical and physical methods. For nanoparticles synthesis using plant can be advantageous over other biological processes by eliminating the elaborate process of maintaining cell cultures (Shankar et al., 2004). It can be suitably scaled up for large-scale synthesis of nanoparticles. The biological systems can provide a number of metal or metal containing particles in the nanometer size range. Some of the examples, the synthesis of magnetite nanoparticles by magnetotactic bacteria (Lovely et al., 1987), siliceous materials by diatoms (Mann, 1993) and gypsum and calcium carbonate layers by S-layer bacteria (Pum et al., 1999). The synthesis of nanoparticles would benefit from the development of clean, nontoxic and environmentally acceptable ‘‘green chemistry’’ procedures and involving organisms ranging from bacteria to fungi and even plants (Bhattacharya et al., 2005; Sastry et al., 2004. The Verticillium sp. and Fusariumoxysporum biomass fungal biomass when exposed to aqueous $AgNO_3$ solution resulted in the intracellular formation of silver nanoparticles and extracellular silver nanoparticles (Senapati et al., 2004). Microorganisms such as bacteria, yeast, fungi and actinomycetes have been described for the formation of nanoparticles and their applications (Sastryet al., 2003).

3.11.1.Green Synthesis of NP

The production of nanoparticles by living organisms or material of biological originfor example, nanoparticles may be synthesized using living bacteria or fungi, or using plant extracts. That techniques provided an advantages over more traditional methods of synthesizing nanoparticles because they are environmentally friendly, can take place around room temperature or lower, and require little intervention or input of energy. In these methods, organisms involved are generally easily cultured in simple organic media, are a renewable resource, and can usually simply be left to do their work. Various organisms could synthesize inorganic particles, including silica and calciumcarbonate, or chalk, that has long been known. Many more microorganisms are able to reduce metal ions to metal. Several bacteria can produce magnetic material by the reduction of iron compounds, incorporating magnetic nanoparticles into bodies known as magnetosomes within their cells. Some types of bacteria have been successfully employed in the biosynthesis of nanoparticles. This can take place both intracellularly and extracellularly. It is thought that the bacteria use the nitrate anion (NO_3^-) as a source of nitrogen, leaving metallic silver.Fungi and flowering plants been used experimentally for synthesize nanoparticles.

3.11.2. Use of plants to synthesize nanoparticles

Group of researchers developed silver nanoparticles being extensively synthesized using various Plant.Different types of plants are being currently investigated for their role in the synthesis of nanoparticles. Gold nanoparticles with a size range of 2- 20 nm have been synthesized using the live alfa alfa plants (Torresday*et al*., 2002). Nanoparticles of silver, nickel, cobalt, zinc and copper have also been synthesized inside the live plants of *Brassica juncea* (Indian mustard), *Medicago sativa* (Alfa alfa) and *Heliantusannus* (Sunflower). Certain plants are known to accumulate higher concentrations of metals compared to others and such plants are termed as hyper-accumulators. Of the plants investigated, *Brassica juncea* had better metal accumulating ability and later assimilating it as nanoparticles (Bali *et al*., 2006). Recently much more work has been done with regard to plant assisted reduction of metal nanoparticles and the respective role of phytochemicals. The main phytochemicals responsible have been identified as terpenoids, flavones, ketones, aldehydes, amides and carboxylic acids in the light of IR spectroscopic studies. The main water soluble phytochemicals are flavones, organic acids and quinones which are responsible for immediate reduction. The phytochemicals present in *Bryophyllum sp*. (Xerophytes), *Cyprussp.* (Mesophytes) and *Hydrilla sp.* (Hydrophytes) were studied for their role in the synthesis of silver nanoparticles. The Xerophytes were found to contain emodin, an anthraquinone which could undergo redial tautomerization leading to the formation of silver nanoparticles. The three types of Mesophyt were studied benzoquinones, namely, cyperoquinone, dietchequinone, and remirin. It was suggested that gentle warming followed by subsequent incubation resulted in the activation of quinones leading to particle size reduction. Catechol and protocatechaldehyde were reported in the hydrophyte studied along with other phytochemicals. It was reported that catechol under alkaline conditions gets transformed into protocatechaldehyde and finally into protocatecheuic acid. Both these processes liberated hydrogen and it was suggested that it played a role in the synthesis of the nanoparticles. The size of the nanoparticles synthesized studies were using xerophytes, mesophytes and hydrophytes were in the range of 2- 5nm (Jha *et al*., 2009). Recently some gold nanoparticles have been synthesized using the extracts of *Magnolia kobus* and *Diopyros kaki* leaf extracts. It was investigated that the effect of temperature on nanoparticle formation was investigated and polydisperse particles with a size range of 5- 300nm were obtained at lower temperature while a higher temperature supported the formation of smaller and spherical particles (Song *et al*., 2009).

3.11.3.Biosynthesis of nanoparticles by algae

The review of literature revealed that the synthesis of nanoparticles using algae as source has been unexplored and underexploited. Recently, there are few, reported that algae being used as a biofactory for synthesis of metallic nanoparticles, which implemented an efficient approach for synthesis of stable gold nanoparticles by the reduction of aqueous $AuCl_4$ using *Sargassum wightii (*Singaravelu et al).

3.11.4. Nanoparticle synthesis using bacteria

Synthesis of metal nanoparticles by using of microbial cells has emerged as a novel approach. Recently, the efforts directed towards the biosynthesis of nanomaterials, the interactions between microorganisms and metals have been well documented and the ability of microorganisms to extract and/or accumulate metals is employed in commercial biotechnological processes such as bioleaching and bioremediation (Gericke& Pinches, 2006). Bacteria are well known to produce inorganic materials either intracellularly or extracellularly. Microorganisms are concluded as a potential biofactory for the synthesis of nanoparticles like gold, silver and cadmium sulphide. Some known examples of bacteria synthesizing inorganic materials include magnetotactic bacteria (synthesizing magnetic nanoparticles) and S layer bacteria which produce gypsum and calcium carbonate layers (Shankar *et al*., 2004).Some types of microorganisms stay alive at high metal ion concentration due to their resistance to the metal. That mechanism involved: efflux systems, alteration of solubility and toxicity via reduction or oxidation, biosorption, bioaccumulation, extra cellular complication or precipitation of metals and lack of specific metal transport systems (Husseiny*et al*., 2007). For example of *Pseudomonas stutzeri* AG 259 isolated from silver mines has been shown to produce silver nanoparticles (Mohanpuria *et al*., 2007). Several microorganisms are known to produce nanostructured mineral crystals and metallic nanoparticles with properties similar to chemically synthesized materials, while exercising strict control over size, shape and composition of the particles. Some examples are, the formation of magnetic nanoparticles by magnetotactic bacteria, the production of silver nanoparticles within the periplasmic space of *Pseudomonas stutzeri* and the formation of palladium nanoparticles using sulphate reducing bacteria in the presence of an exogenous electron donor (Gericke&Pinches, 2006). Though it is widely believed that the enzymes of the organisms play a major role in the bioreduction process, some studies have indicated it otherwise. Some studies are indicated, that some microorganisms could reduce silver ions where the processes of bioreduction were probably non enzymatic. For an example, dried cells of *Bacillus megaterium* D01, *Lactobacillus sp*. A09 were shown to reduce silver ions by the interaction of the silver ions with the groups on the microbial cell wall (Fu *et al*., 1999, 2000). Mainly, silver nanoparticles in the size range of 10- 15 nm were produced by treating dried cells of *Corynebacterium sp*. SH09 with diammine silver complex. The ionized carboxyl group of amino acid residues and the amide of peptide chains were the main groups trapping $(Ag(NH_4))^{2+}$ onto the cell wall and some reducing groups such as aldehyde and ketone were involved in subsequent bioreduction, but it was found that the reaction progressed slowly and could be accelerated in the presence of OH- (Fu *et al*., 2006). In the most cases of bacteria, most metal ions are toxic and therefore the reduction of ions or the formation of water insoluble complexes is a defense mechanism developed by the bacteria to overcome such toxicity (Sastry*et al*., 2003).

3.11.5. Use of fungi to synthesize nanoparticles

Several Fungi has been widely used for the biosynthesis of nanoparticles and the mechanistic aspects governing the nanoparticle formation have also been documented for a few of them. In addition to monodispersity nanoparticles can be obtained using fungi as Compared to bacteria, fungi could be used as a source for the production of large amount of nanoparticles. Yeast, belonging to the class ascomycetes of fungi has shown to have good potential for the synthesis of nanoparticles. Gold nanoparticles have been synthesized using the fungi *V. luteoalbum*. Here, the rate of particle formation and therefore the size of the nanoparticles could an extent be manipulated by controlling parameters such as pH, temperature, gold concentration and exposure time. The extracellular secretion of the microorganisms offers the advantage of obtaining large quantities in a relatively pure state, free from other cellular proteins associated with the organism with relatively simpler downstream processing. The hypothesis indicated that proteins, polysaccharides and organic acids released by the fungus were able to differentiate different crystal shapes and were able to direct their growth into extended spherical crystals (Balaji*et al*., 2009). This indicates that probably the reductases in *F. moniliformae*were necessary for the reduction of Fe (III) to Fe (II) and not for Ag (I) to Ag (0) (Duran *et al*., 2005). Nanocrystalline zirconia was produced by cationic proteins at room temperature while were similar to silicatein secreted by *F. oxysporum* (Mohanpuria *et al*., 2007). Promising synthesis of nanoparticles appears by the use of specific enzymes secreted by fungi.This would lead to the possibility of genetically engineering microorganisms to over express specific reducing molecules and capping agents and thereby control the size and shape of the biogenic nanoparticles (Balaji *et al*., 2009).

3.11.6. Use of actinomycetes to synthesize nanoparticles

Actinomycetes are microorganism's share some characteristics of fungi and prokaryotes such as bacteria. In an effort to elucidate the mechanism or the processes favouring the formation of nanoparticles with desired features, (Ahmad *et al*. 2003), studied the formation of monodisperse gold nanoparticles by *Thermomonospora sp.* and concluded that extreme biological conditions such as alkaline and slightly elevated temperature conditions were favorable for the formation of monodisperse particles. Based on this hypothesis, alkali tolerant actinomycete, *Rhodococcus sp.*

has been used for the intracellular synthesis of monodisperse gold nanoparticles (Ahmad *et al.* 2003). In this study, it was observed that the concentration of nanoparticles were more on the cytoplasmic membrane. This could have been due to the reduction of metal ions by the enzymes present in the cell wall and on the cytoplasmic membrane.

3.11.7. Use of yeast to synthesize nanoparticles

Industrially important strain of yeast (eukaryotic microorganism) has been found to be a prominent candidate for biological synthesis of quantum semiconductors nanoparticles. For the first time, the biological synthesis of cadmium sulphide (CdS) quantum nanocrystals was produced by the strain of Candida glabrata. The yeast biomass produces the intracellularlly, monodispersed spherical shaped quantum nanocrystallites using cadmium salts and by neutralizing the toxicity of metal ions (metalthiolate complex). The synthesized nanocrystals are now used in quantum semiconductor (Ahmad *et al.,* 2003). The strain of *Schizosaccharomyces pombe* were used to improve the quantity of semiconductor nanocrystals and this strain produces hexagonal lattice structured CdS nanoparticles in mid-log phase (incubated) nano crystals were obtained (*A. M. Fayaz et al*). This finding gives an idea that the greater amount of formation of CdS nanocrystals mainly depends on the nature of growth profile of yeast biomass. The mid exponential phase shows maximum production; at the same time the addition of CdS solution during stationary phase results the decreased and/or there is no formation of nanocrystals. (Williams et al.). That reported the formation of CdS nanocrystals were found at early exponential phase of yeast growth, but this time it was affecting the cellular metabolism of the yeast and resulted in efflux of Cd from the cells. Themechanism involves two different steps, initially, an enzyme named phytochelatin synthase activated to synthesize phytochelatins, this reaction leads to forma low molecular weight metal-thiolate complex and eventually transport complex to across the vacuolar membrane by an ATP-binding cassette-type vacuolar membrane protein (HMT1). Ortiz et al. (*N. Jain et al,*) documented that the addition of sulphide to the metal-thiolate complex in the membrane and that results in formation of high molecular weight PC CdS2-complex that allow them to sequestered into vacuole.(Kowshik et al.). Extracellular production of silver nanoparticles was reported using silver tolerant yeast strainMKY3, which synthesized hexagonal AgNP (2-5nm) in log phase of growth. Recently,yeast biomass has been identified for their ability to produce gold nanoparticles, whereby controlling growth and other cellular activities controlled size and shape of the nanoparticles was achieved (*A. Ahmad, et al.,*).More recently, the strain of *Yarrowia lipolytica*NCIM3589 was found to be a good candidate for synthesis of gold nanoparticles associated with cell wall.The reduction of gold ions occurred in pH dependent manner at pH 2.0; it produced hexagonal and triangular gold crystals due to the nucleation on the cell surfaces (*K. Kalishwaralal et al*).

3.11.8.Use of virus to synthesize nanoparticles

An Eco-friendly microbial synthesis of nanoparticles have been received great attention and extended towards intact biological particles (viruses). The biological molecules are plays a vital role in growth of semiconductor as a template, biologicalmolecules includes, fatty acids, amino acids, and polyphates. For example, by interchanging the ratio of different fatty acids (chain lengths), different nature of CdSe, CdS, and CdTe nanocrystals can be achieved. Similarly the variety of other biological materials are also involves in synthesis of inorganic materials. The other important bio-factories like DNA (A.P. Alivisatos et al,1996) , protein cages(K.K.W. Wong et al, 1998) , biolipid cylinder (D. D. Archibald et al,1993), viroid capsules (T. Douglas et al. 1998), bacterial rapidosomes , S-layers and multicellular superstructures (S. A. Davis et al, 1997) are used as template- mediated production of inorganic nanomaterials and microstructured materials. Interestingly, viral scaffolds were found to be a template for the process of nucleation and assembly of inorganic materials. Certainly, cowpea chloroticmottle virus and cowpea mosaic virus have been used as nucleation cages for the mineralization of inorganic nanomaterials. In addition to this, tobacco mosaic virus (TMV) used as template for the successful synthesis of iron oxides by oxidative hydrolysis, co-crystallization and mineralization of CdS and lead sulphide (PbS) crystalline nanowire, and the synthesis of SiO_2 by sol-gel condension. The process happened with the help of external groups of glutamate and aspartate on the external surfaceof the virus. Peptides capable of nucleating nanocrystal growth have been identified from combinatorial screens and displayed on the surface M13 bacteriophage. A hybrid nanowires (ZnS-CdS) are obtained with a dual peptide virus engineered to express A7 and J140 within the same viral capsid. (S.W. Lee et al. and C. Mao et al., 2003)

3.11.9. Synthesis of Silver by solar irradiation from B. amyloliquefaciens

Silver nanoparticles (AgNPs) were obtained by using $AgNO_3$ solar irradiation of cell-free extracts of *Bacillus amyloliquefaciens*. Many factors like light intensity, extract concentration, and NaCl addition influenced the synthesis of AgNPs. TEM (Transmission electron microscopy) and XRD (X-ray diffraction) analysis confirmed that circular and triangular crystalline AgNPs were synthesized. The potential value of the AgNPs, possibly caused by interaction with proteins likely explains the high stability of AgNPs suspensions. AgNPs showed antimicrobial activity against *Bacillus subtilis* and *Escherichia coli* in liquid and solid medium.

3.12. Application of Silver Nanoparticles

3.12.1. Human Health

The production of nanoparticles has many different effects on human health relative to bulk material (Albrecht, 2006). Increase the biological activity of nanoparticles can be determined beneficial, detrimental or both. Nanoparticles are enough to access to skin, lungs, and brain (Koziara et al., 2003; Oberdorster et al., 2004). Exposure of metalcontaining nanoparticles to human lung epithelial cells generated reactive oxygen species, which lead to oxidative stress and damage of the cells (Limbach et al., 2007; Xiet al., 2006).A study on toxic effects of silver nanoparticles was done,that results show a deposition of particles on organs and severe developmental effects. The biocompatibility and toxicity of silver nanoparticles were exhibited at each development stage by observing single silver nanoparticle inside embryos.

3.12.2. Environment

Silver nanoparticles have a great concern to wastewater treatment utilities and to biological systems. The inhibitory effects of silver nanoparticles on microbial growth were evaluated at a treatment facility using an extant respirometry technique. The nitrifying bacteria were susceptible to inhibition by silver nanoparticles, which could have detrimental effects on the microorganisms in wastewater treatment. The environmental risk of silver nanoparticles was recently investigated by determining released silver from commercial clothing. The sock material and wash water contained silver nanoparticles of 10–500 nm diameter.

3.12.3. Catalytic Action

Due to high surface area and high surface energy predetermine metal nanoparticles for being effective catalytic medium. Growing small particles of silver have been observed to be more effective catalysts than stable colloidal particles. These growing particles catalyzed the borohydride reduction of several organic dyes. The reduction rate catalyzed by growing particles is distinctly faster compared to that of stable and larger silver particles, which are the final products of growing particles. Catalysis is due to efficient particle-mediated electron transfer from the BH4- ion to the dye. The catalytic activity of the particles depends on their size, *E*1/2 of the dye, and the dye-particle interaction (Jana et al., 1999). Catalytic activity of silver nanoparticles can be controlled by its size, as redox potential depends on the nanoparticle size (Jana et al., 1999).

3.12.4. Antimicrobial activity of silver nanoparticles

Due to the non-toxic, safe inorganic antibacterial agent of silver nanoparticles being used for centuries and iscapable of killing about 650 microorganisms that cause diseases (Jeong et al., 2005). Silver has been described as being 'oligodynamic', that is, its ions are capable of causing a bacteriostatic (growth inhibition) or even a bactericidal (antibacterial) impact. Therefore, it has the ability toexert a bactericidal effect at minute concentration (Percivala et al., 2005). It has a significantpotential for a wide range of biological application such as antibacterial agents for antibioticresistant bacteria, preventing infections, healing wounds and anti-inflammatory (Taylor et al.,2005). Silver ions (Ag+) and its compounds are highly toxic to microorganism exhibiting strong biocidal effect on many species of bacteria but have a low toxicity towards animal cells.Bactericidal behavior of nanoparticles is attributed to the presence of electroniceffects that are brought about as a result of change in local electronic structure of the surface dueto smaller sizes. The effects are considered to be contributing towards enhancement ofreactivity of silver nanoparticles surface. Silver in ionic form strongly interacts with thiol groups of vital enzymes and inactivates them. That lead DNA loses its replication ability once the bacteria are treated with silver ions (Morones et al., 2005). Silver nanoparticles destabilize plasma membrane potential and depletion of levels of intracellular adenosine tri-phosphate (ATP) by targeting bacterial membrane resulting in bacterial cell death.Compounds of silver such as silver nitrate and silver sulfadiazine are usedto prevent bacterial growth in drinking water, sterilization and burn care.

3.12.5. Silver acts as odor controlling agent

Due to effective antimicrobial agent activity of silver nanoparticles provide terrific driving force for diffusion. The Silver nanoparticles are assembled with many different shapes, such as spheres, rods, cubes, wires, film, and coatings and can be integrated into a variety of materials like metals, ceramics, polymers, glass, and textiles via fine spraying of silver nanoparticle solution (*R. Senje and I. Illuminat., 2009*). Some Athletic clothing companies have incorporated silver nanoparticles into their products mainly for reduce the bad smell. Many textile industriesinsert silver nanoparticles into its products to allow the particles to attach to the filaments. Once the silver nanoparticles encounter sweat from the human body, they naturally release a low concentration of silver ions into the moist environment.

3.12.6. Silver as Bactericidal agent

Nanometers size of bacteria, use enzymes to metabolize nutrients and create energy in a similar fashion as living organisms. They are unicellular with only one compartment of protein and they store all the elements of the cell. Thus, in order to stop the exponential rate of bacterial replication and disrupt the bacterial enzymes and energy metabolism. Silver ions attack microbes in three different pathways including; respiration, replication, and cell wall synthesis. Silver nanoparticles penetrate the bacterial cell membrane and change their structural composition by interacting with the bacteria's sulfate groups, which are the active site of enzymes. Silver ions disrupt the bacterial enzymes responsible for energy metabolism and electrolyte transport. The lack of enzyme activity ultimately suffocates the bacteria. As an additional means of attack, these powerful silver ions also detached the bacterial replication process by disrupting their DNA backbone finallycreating structural imperfections within the cell's protective layers and speeding the collapse or burst of the bacteria *(L. Geranio et al, 2011*). Therefore, by targeting these three areas, silver ions prevent bacteria proliferation by establishing a defense system, slowing bacterial growth, and eventually killing them.

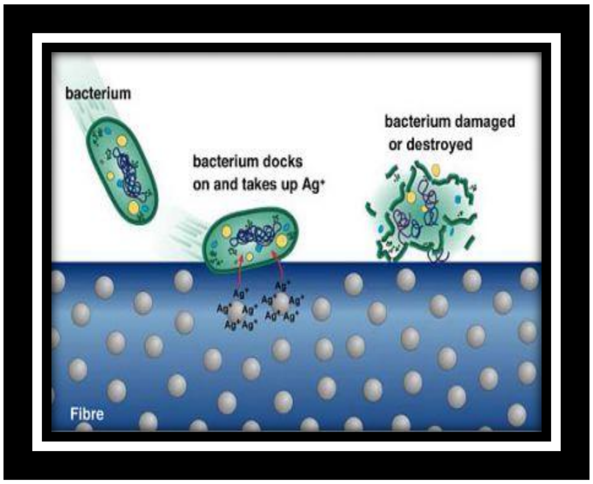


Fig2: *When bacteria take up silver nanoparticles in its cell wall damaged the cell wall of those bacteria and the bacteria are killed.*

4. MATERIALS AND METHODS

4.1. Materials Methods

4.1.1. Chemicals required

Silver nitrate ($AgNO_3$), Nutrient Broth, Nutrient agar wasof analytical grade obtained from Hi media, India.

4.1.2. Glasswares

All glass wares (Conical flasks, Measuring cylinders, Beakers, Petri plates and Test tubes etc.) were purchased from borosil, India.

4.1.3. Strains used

Bacillus thrunigenesis, *Bacillus subtilis, Sthaphylococcus aureus were used to test antimicrobial activity of AgNPs.*

4.2. MIC (Minimum inhibitory concentration) test

MIC is the lowest concentration of an antimicrobial agent that will inhibit the growth of microorganisms after overnight incubation. It is important for the determination or to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. Initially, two bacterial strains like *B. thuringiensis* and *B. susbtilis* were grown in nutrient broth medium and were screened against silver nitrate to find out the minimum concentration of sliver nitrate, which inhibit the growth of bacteria. From the analysis it was found that lowest conc. of $AgNO_3$ given moderate activity against *B. thuringiensis*.

Table2: MIC observation by dilution of AgNO3(50µM)

column	1	2	3	4	5	6	7	8	9	10	11	12
B.T(µl)	5	2.5	1.25	.625	.3	.15	0.078	.04	.02	.01	+ve control	-ve control(5mM AgNO3)
	5	2.5	1.25	.625	.3	.15	.078	.04	.02	.01	+ve control	-vecotrol
B.S(µl)	-vecotrole	5	2.5	125	.625	.3	.15	.078	.04	.02	.01	+ve control
	-vecontrole	5	2.5	125	.625	.3	.15	.078	.04	.02	.01	+vecontrole

4.3. Synthesis of silver nanoparticles from *Bacillus Thuringiensis*

For synthesis of silver nanoparticles the bacterial strain *B. thuringiensis* was inoculated in nutrient broth media along with 35 µM $AgNO_3$. The culture flask was kept on a shaker with speed of 150 rpm at room temperature for overnight incubation. After overnight Incubation the color was changed and turbid was occurred, that indicated the presence of silver nanoparticles in the culture.

4.4. Characterization of silver nanoparticles

Several techniques are used for characterizing different nanoparticles. Here we have discussed the basic principles of few techniques that have been used for the characterized the silver nanoparticles in this project work. They are Absorption spectrophotometer (UV-VIS), Particle size analyzer, X-Ray diffraction (XRD) and Scanning electron Microscope (SEM) and Fourier transforms infrared spectroscopy.

4.4.1. UV-Visible Spectroscopy

According to this technique many molecules absorb ultraviolet or visible light. The percentage of transmittance light radiation determines when light of certain frequency is passed through the samples. This spectrophotometer analysis records the intensity of absorption (A) or optical density (O.D) as a function of wavelength. Absorbance is directly proportional to the path length, L, and the concentration, *c*, of the absorbing species. *Beer's Law* states that:

$$A=€\, C\, L$$

Where € is a constant of proportionality, called the *absorbtivity coefficient*.

4.4.2. SEM

SEM is the scanning electron microscope that creates various images by focusing a high energy beam of electrons onto the surface of a sample and detecting signals from the interaction of the incident electron with the sample's surface. SEM images have greater depth of field yielding a characteristic 3D appearance useful for understanding the morphology material. Magnification is of order 10,000 X and resolution 10 nm.

Scanning process:

- In electron surface when streamof electrons is formed and accelerated towards the specimen using positive electric potential.
- Using metal apertures and magnetic lenses the stream confined and focused into a thin focused monochromatic beam.
- In sample using a magnetic lens the focusing beam create interactions inside the irradiated sample affecting the electron beam.

4.4.3. XRD

- In XRD a large fraction of the X-rays that are not simply absorbed or transmitted by the object but are scattered.
- When an X-ray beam hits an atom, the electrons around the atom start to oscillate with the same frequency as the incoming beam creating an electric field. All directions have

destructive interference, that is, the combining waves are out of phase and there is no resultant energy leaving the solid sample.

- However the atoms arranged like regular pattern in a crystal, and in a very few directions we will have constructive interference. The waves will be in phase and there will be well defined X-ray beams leaving the sample at various directions.
- Hence, a diffracted beam may be described as a beam composed of a large number of scattered rays mutually reinforcing one another.
- X-ray diffraction provides a useful tool to study the structure and composition of the materials which is a key requirement for understanding materials properties.

4.4.4. FTIR

FTIR (Fourier Transform Infra-red Spectroscopy) is a sensitive technique useful for identifying organic chemicals in a whole range of applications although it can also characterize some inorganic include paints, adhesives, resins, polymers, coatings and drugs. It is powerful tool for isolating and characterizing organic contamination.

FTIR is the fact that the most molecules absorb light in the infra-red region of the electromagnetic spectrum. This absorption corresponds to the bonds present in the molecule. The frequency range is measured as wave numbers typically over the range 4000 – 600 cm^{-1}. The background emission spectrum of the IR source is first recorded, followed by the emission spectrum of the IR source with the sample in place. The ratio of the sample spectrum to the background spectrum is directly related to the sample's absorption spectrum. The resultant absorption spectrum from the bond natural vibration frequencies indicates the presence of various chemical bonds and functional groups present in the sample. FTIR is particularly useful for identification of organic molecular groups and compounds due to the range of functional groups, side chains and cross-links involved, all of which will have characteristic vibration frequencies in the infra-red rang.

4.5. Antimicrobial activity test

4.5.1. Preparation of sample with silver nanoparticles

The antimicrobial activity of synthesized nanoparticles was studied by cfu (Colony Forming Unit) measurements against some pathogenic bacteria like *Bacillus. subtilis, staphylococcus aureus and* Escherichia coli. Required amount of agar medium was prepared along with silver nanoparticles of concentration 100µg/ml. Another agar media without silver nanoparticles was also prepared which was used for culturing strains whithout nanoparticles. Both were autoclaved and poured into different petriplates. The above strains were cultured to the petriplated by spread plate methods.The cultures without nanoparticles were kept as control for a comparison study.

5. RESULTS

5.1. Results and discussions

5.1.1. MIC Test:

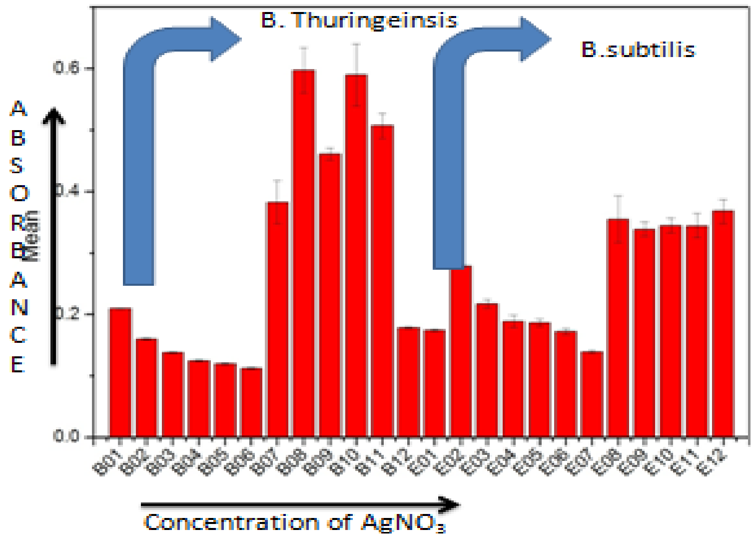


Fig3: MIC result of $AgNO_3$ against B.thuringiensis and B.subtilis

[The fig3 contained X-Axis = Concentration of $AgNO_3$ and Y-axis = Optical Density (absorbance)]

The MIC test was performed to find out the minimum inhibitory concentration of $AgNO_3$ against two organisms. According to the fig-3 the MIC results shows that lowest conc. Of AgNO3 gives moderate activity against *B. thuringiensis*. So we have taken *B. thuringiensis* as potential bacteria for synthesis of silver nanoparticles.

5.1.2. SEM

The fig-4 shown below represent the SEM analysis of *B. thuringiensis* along with nanoparticles prepared by fixing method the fig- shows that most of the nanopaticles are on the surface of bacterial cell wall. So we may suggest that the enzymes responsible for synthesis of silver nanoparticles may be presents in the cell wall of bacteria.

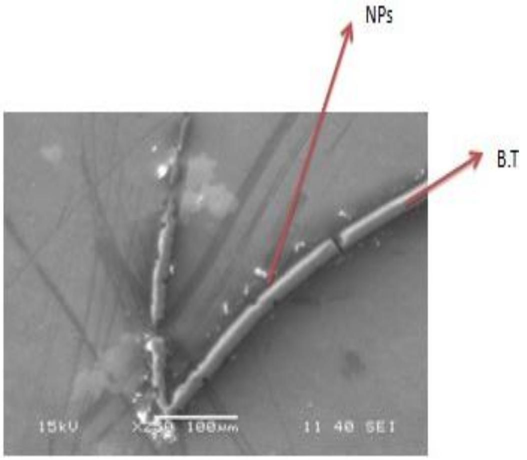


Fig4: SEM analysis of Ag nanoparticles synthesis on the surface B. thuringiensis

5.2. Synthesis of silver nanoparticles

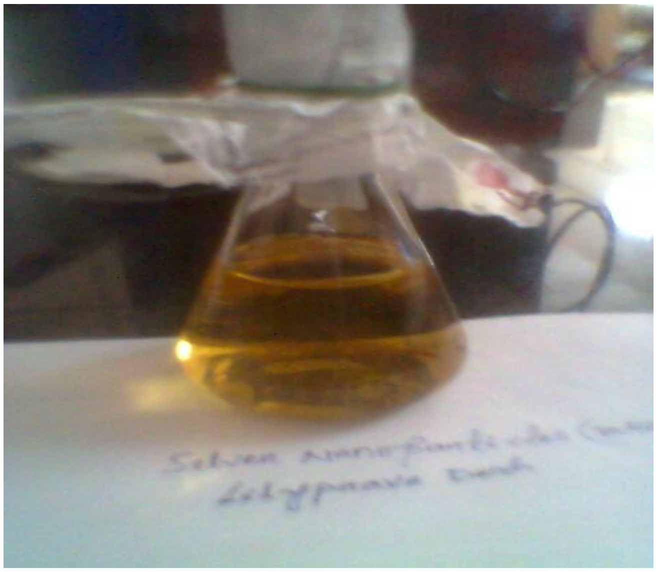


Fig5: *Synthesis of silver nanoparticles by B. thuringiensis.*

Fig5- shows the over-night culture of ***B. thuringiensis*** along with $AgNO_3$ of concentration 35 µM. Color of the solution clearly confirm the reduction of $AgNO_3$ by *B. thuringiensis* forming silver nanoparticles.

5.3. Characterization of silver nanoparticles

5.3.1. UV-Vis spectroscopy Analysis

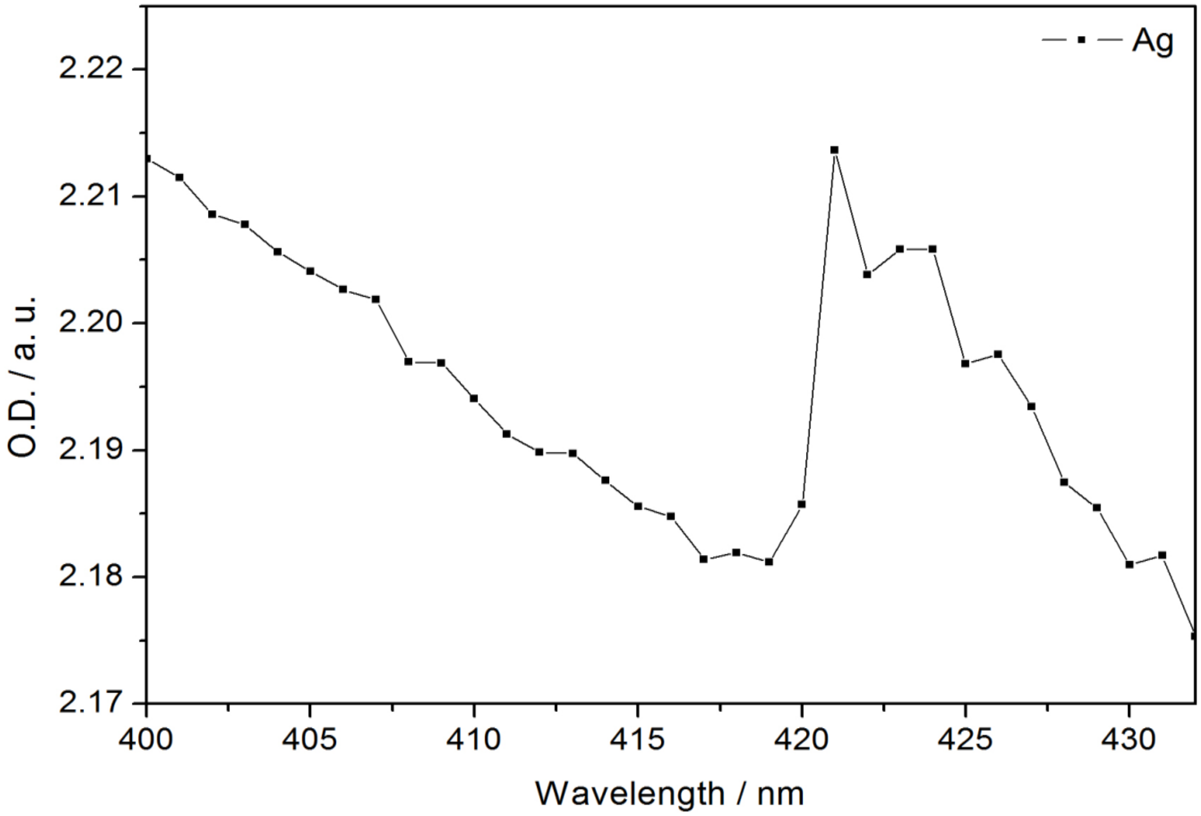


Fig 6: *UV-Vis spectra of silver nanoparticles synthesized byB.thuringiensis*

Fig6- shows the UV-Vis spectra of silver nanoparticles synthesized by *B. thuringiensis*. The peak of the above spectra was found at 421nm and this peak is due to Surface Plasmon Resonance (SPR) property of silver nanoparticles.

5.3.2. SEM Analysis

The morphological features of synthesized silver nanoparticles were studied by SEM analysis shown in fig-7 (below). SEM analysis suggested that most of the particles are spherical in shape.

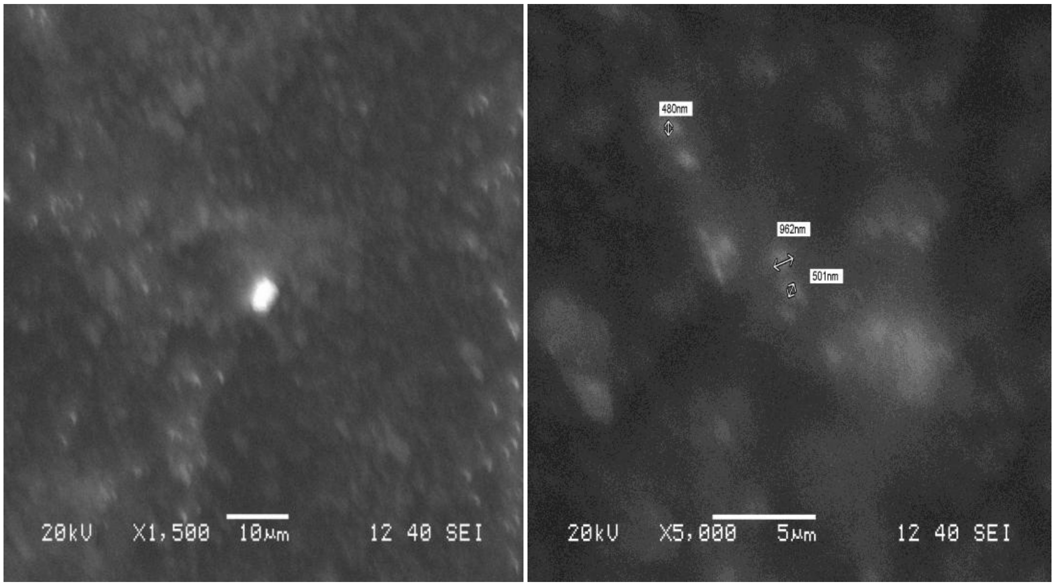


Fig7: SEM image of silver nanoparticles synthesize by *B. thuringiensis*

5.3.3. XRD Analysis

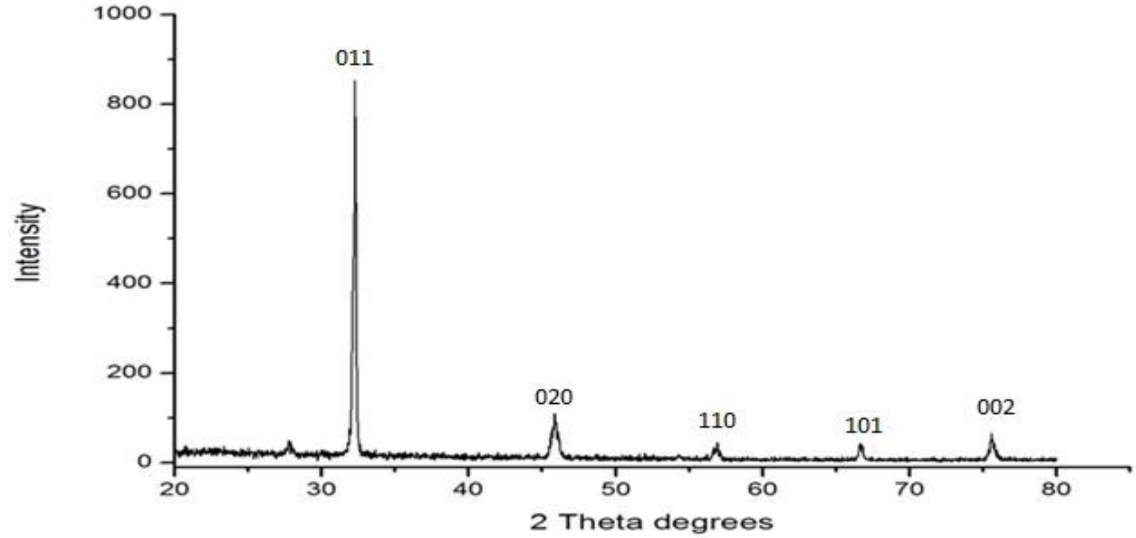


Fig8: XRD spectra of silver nanoparticles

XRD spectrum of synthesized silver nanoparticles (Fig.8) shows distinct diffraction peaks arround 32^0. These sharp Bragg peaks might have resulted due to crystalline nature of silver nanoparticles.

5.3.4. FTIR Analysis.

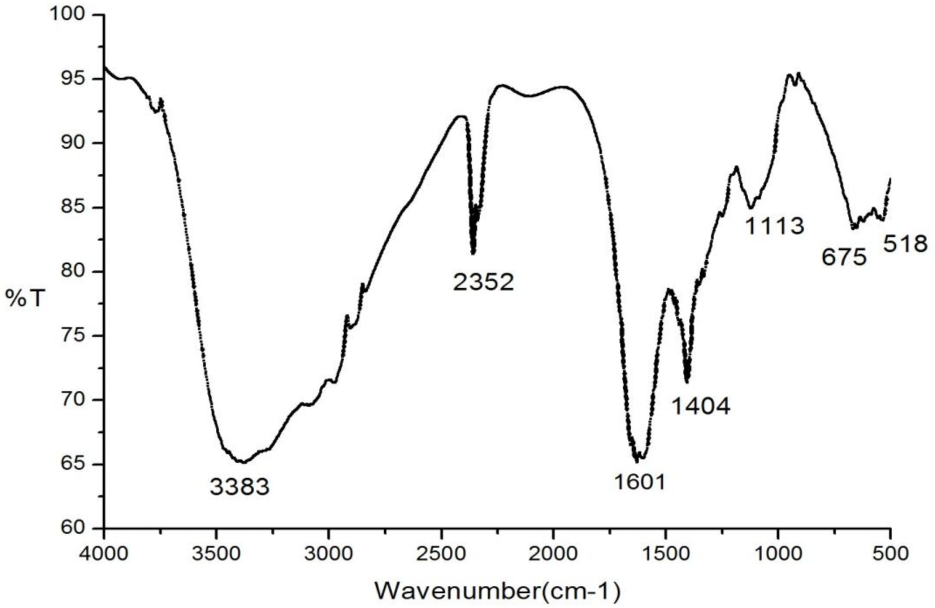
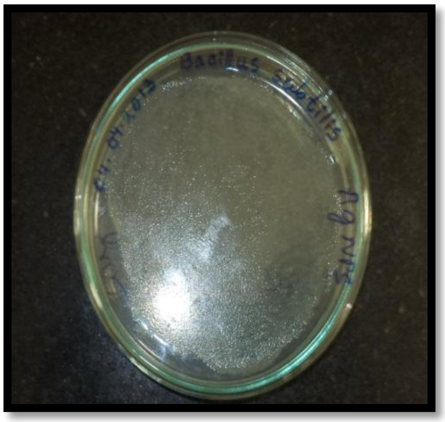
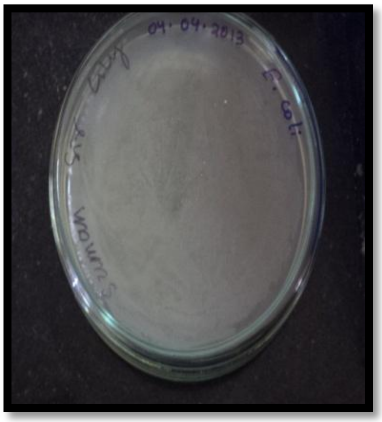


Fig9: ***FTIR spectra from solid powder silver nanoparticles***

The Fig 9-shows the FTIR image of silver nanoparticles synthesized from *B. thuringiensis* .FT-IR analysis revealed the strong bands at 3383, 2352, 1601, and 1404,1113, 675, 518 cm^{-1}. The band at 2352 for O-H stretching corresponds to carboxylic acid, 1601 cm^{-1} for stretching C=C corresponds to aromatic amino groups. The band at 675 cm^{-1} corresponds to C-H stretching of phenyl ring of substitution band, whereas the stretch for Ag-NPs was found around 518 cm^{-1}.

5.4. Antibacterial activity test:





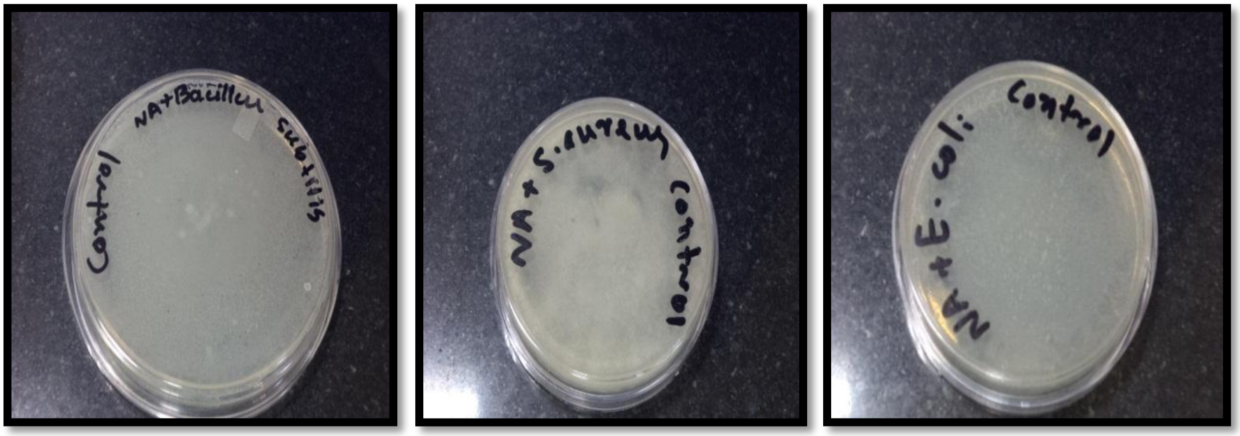


Fig10: *Antibacterial activity of silver nanoparticles against B. subtilis, S. aureus, E. coli*

The Fig10- shows the antibacterial activity of silver nanoparticle against *B. subtilis, S. aureus, E. coli.* According to the figure-10, the growth of S. aureus and B. subtilis and E. coli is inhibited in the presence of nanoparticles as compared control. From the results, we can conclude that silver nanoparticles have significant antibacterial activity against *B. subtilis* and *S. aureus* whereas less against *E. coli.*

6. CONCLUSION

Silver nanoparticle was successfully synthesizing by biological method from *Baccilus thringiensis. T*he Surface Plasmon Resonance (SPR) property of synthesized nanoparticle was studied by UV-Vis spectroscopy and the peak of the spectra was found to be at 421 nm. The morphological study of AgNPs using SEM suggests that the nanoparticles are spherical in shape with diameter around 450 nm to 1000 nm. The physiochemical properties of silver nanoparticles using FTIR, XRD conclude that the nanoparticle form in the process is crystalline with miller index of 011 and angle of diffraction of $2\theta = 32^{O}$. The antibacterial activity of silver nanoparticles concludes that the silver nanoparticles shows significant antibacterial activity against *B. subtilis* and *S. aureus* whereas less activity against *E. coli.*

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