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Research Review

Biosynthesis of Silver Nanoparticles by some Microorganisms Isolated from Jewelry Shop Waste

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Abstract

The organisms were isolated from Jewelry molded waste and used for silver nanoparticle synthesis. The all isolated which was synthesizing silver nanoparticles were studied thoroughly. Synthesized silver nanoparticles were characterized by using nanoparticle tracking analysis and X-Ray diffraction measurements. The effect of various parameters in the synthesis of silver nanoparticles with respect to temperature, salt concentration stability was evaluated. The isolates in 1mM showed temperature stability SPS1 in between 20^0 C to 37^0C while SPL1, HPS 1, HWP1, were found to more efficient at 37^0C. The isolates in 2mM $AgNO_3$ concentration showed temperature optima in between 10^0C to 37^0C. In case of salt concentration effect 0.5 to 2.0 % salt concentration was used. The salt was added when the growth was observed in the form of turbidity followed by addition of 1mM and 2mM $AgNO_3$ solution and incubated at 37°C for 24 hrs.

Key words: Nanoparticles, X-Ray diffraction, Concentration

Nanotechnology is the art and science of manipulating of matter at the nanoscale (down to 1/100000 the width of human hair) to create new and unique materials and products with enormous potential to change the society [www.nanotechproject.org] 1 nanometer (nm) = 1 billionth of the meter.

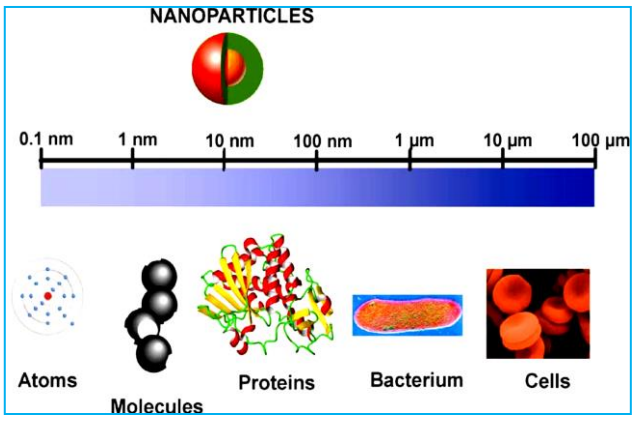

Fig 1 Size of nanoparticle

This particle has nominal diameter like that geometric aerodynamic, mobility, projected area or otherwise of 100 nano, eters or less is the ISO (International Standards Organization) of nano particles [$107 at ANSI eStore at http://webstore.ansi.org].

Nanotechnology speaks like a driving force in new industrial revolution in both private and public sector constantly increasing the spending. In public research spending has reached level over EUR 3 world wild in privet sector spending is even faster – expected to exceed government spending in 2005. It is the major technological force to change shape of Allianz's business environment for all industrial sectors in the seeable future and to deliver substantial growth opportunities. The nanotechnology product size as compare to biological sector, while the expected growth rates over next few years are far higher.

In the market point of view, it performs on nanometer scale with widespread application to enabling technology in various industries. Nanotechnology encompasses the production and also applications of physical, chemical and biological systems at scales ranging from individual atoms or molecules 100 nanometers. As well as the integration of the resulting nano structures into larger systems. The area of this

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dot of "I" alone can encompass 1 million nanoparticles. Nanoscale material are used to have decade application like window glass and sunglass to car bumpers and paints.

Nanotechnology-make its different?

A nanometer is thousand millionth of a meter. A single human hair is about 80,000 nm wide. The RBCs is approximately 7,000 nm wide; the DNA molecule is 2 to 2.5 nm while water molecules 0.3 nm. The term "nanotechnology" was created by Norio Taniguchi of Tokyo University in 1974 to describe to describe the precision manufacture of materials with nanometer tolerances [1] but its origin date back to Richard Feynman's in 1959 talk "there's plenty of room at the bottom [http://www.zyvex.com/nanotech/feynman.html]. In that he proposed that direct manipulation of individual atoms is more powerful in synthetic chemistry. Eric Drexler of MIT expands taniguchis definition and popularized nanotechnology in his 1986 book "Engines of Creation: The coming era of Nanotechnology" [http://www.foresight.org/EOC/]. From below 100nm down to the size of atom the properties of materials can be very different from in larger scale. Nanoscience is the study of phenomena and manipulation of materials at atomic, molecular and macromolecular scales to understand and exploit properties that differ significantly from in larger scale. It is the design characterization, production and application of structures, devices and systems by controlling shape and size on a nanometer scale.

The year 1930s is the modern industrial nanotechnology origin by used to made silver coating for photographic film, chemists make polymers which are a large molecule made by nano scale sub units, in many decades. the earliest use of nanoparticles in ninth century during the Abbasid dynasty. These nanoparticles are used in Arab potters for their glazes therefore the object would change colour depending on view of angle for that called polychrome luster. [http://www.newmaterials.com/news/680.asp]. Today's nanotechnology, are the planned manipulation of materials and properties on a nano scale and exploits the interaction of three technological streams [http ://www.wtec.org/loyola/nano].

1. New and improved control of the size and manipulation of nano scale building blocks.
2. New and improved characterization on nanoscales (e.g., spatial resolution, chemical sensitivity).
3. New and improved understanding of the relationships between nanostructures and properties and how these can be engineered.

The industrialization and urbanization the environment is totally damaged. The dangerous chemicals, gases substances are released for that we learned about the secrets which are present in environment. These nature products have growth advancements in the synthesis process of nanoparticles. The application is highly suitable to biological molecules in a highly controlled assembly for making them suitable nanoparticles synthesis which reliable and ecofriendly [Harekrishna Bar et al. 2009]. The semiconductor metal nanoparticles synthesis is a vast area of research due to potential application it was implemented in development of novel technologies [Cassandra et al]. The nanotechnology is an upcoming area in the modern field of material science. They show completely new or improved properties like that size, morphology and distribution etc. In various field the novel application of nanoparticles and non-materials are emerging out [2].

The medical properties of silver have been known for over 2000 years. Since the nineteenth century, silver-based compounds have been in many antimicrobial applications. Nanoparticles have been known to be used for numerous physical, biological and pharmaceutical applications. Silver nanoparticles are being used as antimicrobial agents in many public places such as railway station and elevators in China, and they are said to show good antimicrobial action.

It is a well-known fact that silver ions and silver-based compounds are highly toxic to microorganism which include 16 major species of bacteria [3-4]. This aspect of silver makes it an excellent choice for multiple roles in the medical field. Silver is generally used in the nitrate form to include antimicrobial effect, but when silver nanoparticles are used, there is huge increase in the surface are available for the microbe to be exposed to. Though silver nanoparticles find in many antibacterial applications the action of this metal on microbes is not fully known. it has been hypothesized that silver nanoparticles can cause cell lysis or inhibit cell transduction. There are many mechanisms involved in cell lysis and growth inhibition.

There are many ways depicted in various literatures to synthesize silver nanoparticles. This includes physical, chemical, and biological methods. The physical and chemical methods are numerous in number, and many of these methods are expensive or use toxic substances which are major factors that make them 'not so favored' methods of synthesis. An alternate, feasible method to synthesis silver nanoparticles is to employ biological methods of using microbes and plants.

Silver nanoparticles find use in many fields, and the major applications include their use as catalysts, as optical sensor sofzeptomole (10^{-21}) concentration, in textile engineering, in electronics, in optics, and most importantly in the medical field as a bactericidal and as a therapeutic agent. Silver ions are used in the formulation of dental resin composites; in coatings of medical devices; as a bactericidal coating in water filters; as an antimicrobial agent in air sanitizer sprays, pillows, respirators, socks, wet wipes, detergents, soaps, shampoos, toothpastes, washing machines, and many other consumer products; as bone cement; and in many wound dressings to name a few. Though there are various benefits of silver nanoparticles, there is also the problem of nano toxicity of silver. There are various literatures that suggest that the nanoparticles can cause various environmental and health problems, though there is a need for more studies to be conducted to conclude that there is a real problem with silver nanoparticles.

This review provides an idea of the antimicrobial properties silver possesses as a nanoparticle, the various methods employed to synthesize silver nanoparticles, and an overview of their applications in the medical field and also discusses the toxicity of silver nanoparticles. The focus is on the characteristics of silver nanoparticles which make them excellent candidates for use in the medical field besides delving into the unique ability of certain biological systems to synthesize silver nanoparticles and also look at the chances of these particles to induce toxicity in humans and the environment as a whole.

Action of silver nanoparticles on microbes

The exact mechanism which silver nanoparticles employ to cause antimicrobial effect is not clearly known and is a debated topic. There are however various theories on the action of silver nanoparticles on microbes to cause the microbicidal effect. Silver nanoparticles have the ability to anchor to the bacterial cell wall and subsequently penetrate it, thereby causing structural changes in the cell membrane like the permeability of the cell membrane and death of the cell. There is formation of 'pits' on the cell surface, and there is accumulation of the nanoparticles on the cell surface [5]. The

formation of free radicals by the silver nanoparticles may be considered to be another mechanism by which the cells die. There have been electron spin resonance spectroscopy studies that suggested that there is formation of free radicals by the silver nanoparticles when in contact with the bacteria, and these free radicals have the ability to damage the cell membrane and make it porous which can ultimately lead to cell death [6-7]. It has also been proposed that there can be release of silver ions by the nanoparticles [8], and these ions can interact with the thiol groups of many vital enzymes and inactivate them [9]. The bacterial cells in contact with silver take in silver ions, which inhibit several functions in the cell and damage the cells. Then, there is the generation of reactive oxygen species, which are produced possibly through the inhibition of a respiratory enzyme by silver ions and attack the cell itself. Silver is a soft acid, and there is a natural tendency of an acid to react with a base, in this case, a soft acid to react with a soft base [10]. The cells are majorly made up of sulfur and phosphorus which are soft bases. The action of these nanoparticles on the cell can cause the reaction to take place and subsequently lead to cell death. Another fact is that the DNA has sulfur and phosphorus as its major components; the nanoparticles can act on these soft bases and destroy the DNA which would definitely lead to cell death [11]. The interaction of the silver nanoparticles with the sulfur and phosphorus of the DNA can lead to problems in the DNA replication of the bacteria and thus terminate the microbes. It has also been found that the nanoparticles can modulate the signal transduction in bacteria. It is a well-established fact that phosphorylation of protein substrates in bacteria influences bacterial signal transduction. Dephosphorylation is noted only in the tyrosine residues of gram-negative bacteria. The phosphotyrosine profile of bacterial peptides is altered by the nano particles. It was found that the nanoparticles dephosphorylate the peptide substrates on tyrosine residues, which leads to signal transduction inhibition and thus the stoppage of growth. It is however necessary to understand that further research is required on the topic to thoroughly establish the claims [12].

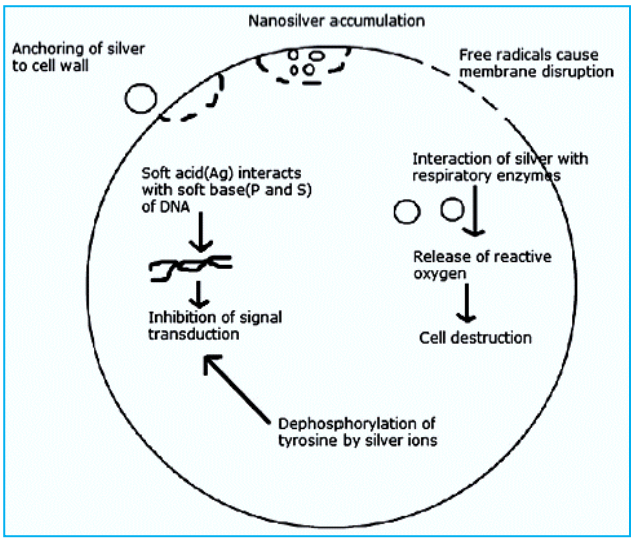


Fig 2 Various modes of action of silver nanoparticles on bacteria

Biological synthesis of silver nanoparticles

The problem with most of the chemical and physical methods of nano-silver production is that they are extremely expensive and also involve the use of toxic, hazardous chemicals, which may pose potential environmental and biological risks. It is an unavoidable fact that the silver nanoparticles synthesized have to be handled by humans and must be available at cheaper rates for their effective utilization; thus, there is a need for an environmentally and economically feasible way to synthesize these nanoparticles. The quest for such a method has led to the need for biomimetic production of silver nanoparticles whereby biological methods are used to synthesize the silver nanoparticles. The growing need to develop environmentally friendly and economically feasible technologies for material synthesis led to the search for biomimetic methods of synthesis [13]. In most cases, the chemical synthesis methods lead to some chemically toxic substances being absorbed on the surface and can hinder their usage in medical applications [14]. There are three major sources of synthesizing silver nanoparticles: bacteria, fungi, and plant extracts. Biosynthesis of silver nanoparticles is a bottom-up approach that mostly involves reduction/oxidation reactions. It is majorly the microbial enzymes or the plant phytochemicals with antioxidant or reducing properties that act on the respective compounds and give the desired nanoparticles. The three major components involved in the preparation of nanoparticles using biological methods are the solvent medium for synthesis, the environmentally friendly reducing agent, and a nontoxic stabilizing agent. The first evidence of bacteria synthesizing silver nanoparticles was established using the *Pseudomonas stutzeri AG259* strain that was isolated from silver mine [15]. There are some microorganisms that can survive metal ion concentrations and can also grow under those conditions, and this phenomenon is due to their resistance to that metal. The mechanisms involved in the resistance are efflux systems, alteration of solubility and toxicity via reduction or oxidation, biosorption, bioaccumulation, extracellular complex formation or precipitation of metals, and lack of specific metal transport systems [16]. There is also another aspect that though these organisms can grow at lower concentrations, their exposure to higher concentrations of metal ions can induce toxicity. The most widely accepted mechanism of silver biosynthesis the presence of the nitrate reductase enzyme. The enzyme converts nitrate into nitrite. In in vitro synthesis of silver using bacteria, the presence of alpha-nicotinamideadenine dinucleotide phosphate reduced form (NADPH)-dependent nitrate reductase would remove the downstream processing step that is required in other cases. During the reduction, nitrate is converted into nitrite and the electron is transferred to the silver ion; hence, the silver ion is reduced to silver (Ag^+ to Ag^0). This has been said to be observed in *Bacillus licheniformis* which is known to secrete NADPH and NADPH-dependent enzymes like nitrate reductase that effectively converts Ag^+ to Ag^0 [17]. The mechanism was further confirmed by using purified nitrate reductase from *Fusarium oxysporum* and silver nitrate along with NADPH in a test tube, and the change in the color of the reaction mixture to brown and further analysis confirmed that silver nanoparticles were obtained [18]. There are also cases which indicate that there are other ways to biosynthesize silver nanoparticles without the presence of enzymes. It was found that dried cells of *Lactobacillus sp.* A09 can reduce silver ions by the interaction of the silver ions with the groups on the microbial cell wall [19].

Silver-synthesizing fungi

When in comparison with bacteria, fungi can produce larger amounts of nanoparticles because they can secrete larger amounts of proteins which directly translate to higher productivity of nanoparticles [20]. The mechanism of silver nanoparticle production by fungi is said to follow the following steps: trapping of Ag^+ ions at the surface of the fungal cells and the subsequent reduction of the silver ions by the enzymes

present in the fungal system [21]. The extracellular enzymes like naphthoquinones and anthraxquinones are said to facilitate the reduction. Considering the example of *F. oxysporum*, it is believed that the NADPH-dependent nitrate reductase and a shuttle quinine extracellular process are responsible for nanoparticle formation [22]. Though the exact mechanism involved in silver nanoparticle production by fungi is not fully deciphered, it is believed that the above-mentioned phenomenon is responsible for the process. A major drawback of using microbes to synthesize silver nanoparticles is that it is a very slow process when in comparison with plant extracts. Hence, the use of plant extracts to synthesize silver nanoparticles becomes an option that is feasible.

Silver-synthesizing plants

The major advantage of using plant extracts for silver nanoparticle synthesis is that they are easily available, safe, and nontoxic in most cases, have a broad variety of metabolites that can aid in the reduction of silver ions, and are quicker than microbes in the synthesis. The main mechanism considered for the process is plant-assisted reduction due to phytochemicals. The Main phytochemicals involved are terpenoids, flavones, ketones, aldehydes, amides, and carboxylic acids. Flavones, organic acids, and quinones are water-soluble phytochemicals that are responsible for the immediate reduction of the ions. Studies have revealed that xerophytes contain emodin, an anthraquinone that undergoes tautomerization, leading to the formation of the silver nanoparticles. In the case of mesophytes, it was found that they contain three types of benzoquinones: cyperoquinone, dietchequinone, and remirin. It was suggested that the phytochemicals are involved directly in the reduction of the ions and formation of silver nanoparticles [23]. Though the exact mechanism involved in each plant varies as the phytochemical involved varies, the major mechanism involved is the reduction of the ions.

Medical applications of silver nanoparticles

Silver nanoparticles, due to their unique properties, find use in many day-to-day applications in human life. A few examples include their addition in house cleaning chemicals, in fabric cleaners, as antireflection coatings, to improve the transfer of heat from collectors of solar energy to their fuel tanks, to produce high-performance delicate electronics, and in hundreds of other applications. Though all these are important applications of silver nanoparticles, perhaps their need is most desired in the medical field. The general aspect of nanoparticles is that the small Size of nanoparticles provides for a larger surface area for the particle and hence increases the effect. The nano size of the particles also increases the penetration potential of the silver particles, hence again aiding in better utilization of the metal properties. Based on the size factor.

Alone, nanoparticles have the ability to penetrate the circulatory system and translocate even the blood–brain barrier in the human system. The antimicrobial nature of silver nanoparticles is the Most exploited nature of silver nanoparticles in the medical field, though the anti-inflammatory nature is also considered immensely useful in the medical field. Initial studies have suggested that the acceleration of wound healing in the presence of nanoparticles is due to the reduction of local matrix metalloproteinase (MMP) activity and the increase in neutrophil apoptosis within the wound. It has been suggested that the MMP can induce inflammation and hence cause non-healing wounds [24]. A reduction in the levels of pro-inflammatory cytokines was also demonstrated in a mouse model with burn injury when silver nanoparticles were introduced [25]. It was also found that silver nanoparticles can inhibit the activities of interferon gamma and tumor necrosis factor alpha which are involved in inflammation [26]. Though these studies prove that silver nanoparticles are involved in the anti-inflammatory effects, the exact, precise mechanism of action remains to be determined. The anti-inflammatory effects induced by nano silver however make it an excellent candidate for use as anti-inflammatory agents that can be used for various therapies. Dr. Robert Burrell is said to have developed the world's first nano silver-based wound dressing in 1995. He developed Acticoat that speeds up the healing process and removes scars if any [27]. Acticoat has become the final word when it comes to wound dressings; however, there are numerous other players in the same field. It is sold worldwide by Smith and Nephew plc. Nano silver is effective due to the fact that it has a much better effect on the bacteria that tend to infect the wound and due to the fact that it can easily penetrate the wound through the body fluids. The most prominent players in silver-based wound healing are Acticoat 7, Acticoat Moisture Control, Acticoat Absorbent, Silvercel, Aquacel Ag, Contreet F, Urgotol SSD, and Actisorb. In July 2010, there were reports that scientists at the University of Bath and the burns team of the South West UK Paediatric Burns Centre at Frenchay Hospital in Bristol were working on an advanced bandage that works by releasing antibiotics from nano capsules triggered by the presence of pathogenic bacteria. The dressing is said to have the potential to change color when the antibiotic is released and hence alerting that there is an infection in the wound. Experts believe that this dressing has great potential in treating burn victims who are susceptible to toxic shock syndrome. With the advent of such system, there can be a reduction in antibiotic resistance [http://nanobiotechnews.com. 2012]. As of 2012, reports suggest that the bandage could be commercialized any time soon. There are newer, efficient, and safer silver nanoparticle-based wound dressings that are being introduced in the market.

Silver nanoparticles are used in bone cements that are used as artificial joint replacements. Polymethyl methacrylate loaded with nano silver is being considered as bone cement as the nano silver can induce antimicrobial activity [28]. Ultrahigh molecular weight polyethylene has been the preferred choice for fabricating inserts for total joint replacement components, but it is susceptible to wear and tear which is a major drawback. To overcome this, silver nanoparticles were added, and the presence of silver nanoparticles drastically reduced the wear and tear of the polymer [29]. The currently used methods to prevent surgical infection include antibiotics and antiseptics. Surgical meshes are used to bridge large wounds and for tissue repairs. Though these meshes are effective, they are susceptible to microbial infections. Silver nano particle coated polypropylene mesh is said to have good antimicrobial activity and can be considered an ideal candidate for surgical meshes [30]. The antimicrobial property of silver nanoparticles is documented, and it has immense potential to be used in disinfectants [31]. It is also believed that most medical treatments such as intravenous catheters, endotracheal tubes, wound dressings, bone cements, and dental fillings can all make use of nano silver to prevent microbial infections. Nano silver also has the capacity to be used in bio sensing. The plasmonic properties of nano-silver are dictated by its shape, size, and the dielectric medium that surrounds it. Its properties in the dielectric medium that can be exploited make it an ideal candidate for biosensing. Nanosilver biosensors can effectively bio sense a large number of proteins that normal biosensors find hard to detect. This unique advantage that nano silver has can be utilized for detecting various abnormalities and diseases in the human body including cancer [32].

The plasmonic properties of nano silver also make it an excellent candidate for bioimaging as they, contrary to commonly used fluorescent dyes, do not undergo photo bleaching and can be used to monitor dynamic events over an extended period of time [33]. The plasmonic nature of nanosilver can also be used to destroy unwanted cells. The cells can be conjugated to the target cells and then be used to absorb light and convert it to thermal energy; the thermal energy can lead to thermal ablation of the target cells [34]. Studies over the years have proven that it is difficult to remove silver completely if deposited in the body. Animal and human studies have indicated that nano silver can be excreted through the hair, urine, and feces majorly [35]. However, the main excretion source is biliary excretion. Once orally administered, silver particles pass through the liver, then into the bile, and is excreted out through feces. In the case of inhalation, the particles enter the lungs and subsequently the blood stream and the other organs and are excreted out through urine or feces. The silver particles can enter the body through the skin too from where they enter the blood stream and are taken to various organs and are finally excreted out through urine or feces.

The nanoparticles have lot of importance in the science field. It is produced in various ways as physical, chemical and biological. But as compared to all three methods biological synthesized method is an eco-friendly and size is also controlled.

Therefore, the aim of this study was isolation and characterization of silver nanoparticles synthesizing from Saraf market waste and also in jewelry preparation area which are Sangali and Kolhapur respectively.

In this work we cover isolation of silver nanoparticles synthesizing bacteria and application those with followings objectives:

To collect solid sample.

1) Enrichment and isolation of silver nanoparticles synthesizing bacteria.
2) To grow in broth to produce mass culture.
3) To separate supernatant for extracellular nanoparticles synthesis.
4) To study the given or to used in antibacterial activity.
5) To study the various environmental effects in the synthesis of silver nanoparticles.
6) To study colony characteristics.
7) To study nanoparticles synthesis in a suitable agar nutrient medium.
8) To characterize the silver nanoparticles using various analytic method.

MATERIALS AND METHODS

Sample was collected from the area where jewelry metal molded and washed waste discarded, this soil sample was taken in a sterile polythene bag which was closed tightly and brought to laboratory for preservation in refrigerator till future use. In the time of collection temp of the region was recorded and the color of soil sample observe visually.

Enrichment

Soil samples brought from various sampling sites were added to nutrient broth supplemented with $AgNO_3$ for enrichment. After enrichment for about 48 hours; broths were further used for isolation studies.

Isolation of metal resistance bacteria

The above discussed soil which is contaminated with metals are used as a source of isolation of bacteria species, for that reason the given soil sample was taken 1gm diluted in to 10 ml sterile distilled water.

Then the looped of supernatant was streaked on nutrient agar plate and incubated at ambient temperature, in particular time interval when the colonies formed which are picked up and making a suspension in sterile saline water. Then the loopful of Sample was streaked on agar nutrient medium with 1and2 mM concentrated of $AgNO_3$ added in medium when the colonies are produced in above medium thus are further purified to above obtain pure culture and also mass culture. It also transformed on slant (NA) stored at 4 °C as stocked master culture in refrigerator till their further use.

Characterization of isolates

The given isolated were studied for following characters.

a) *Colony characters*

The suspension was prepared in above maintained Frequency slant and streaked of agar, nutrient medium. Then the plates were incubated at ambient temperature, for particular time of interval then goes to study of colony characters morphological characters, physiological and biological characterization also.

b) *Morphological characterization*

Fresh suspension of each isolate on plates were used to steady Gram nature Hucker –Conn's (1923) gram staining method and motility was done by hanging drop technique.

c) *Physiological characterization*

I) *Temperature requirement*

The effect of temperature on growth and nanoparticles synthesis was carried out by incubating the broth tubes in different temp viz. 10 °C, 20 °C, 26 °C, 37 °C. The nanoparticles synthesis was observed at brown color formation.

II) *Salt requirement*

The effect of salt concentration of growth and nanoparticles synthesis was observed by incubating them into NB of different salt concentrations as 0.5%, 1.0%, 1.5%, 2% after incubating color change (yellow to brown) was observed and result were recorded.

d) *Biochemical characterization*

I) *Hugh and Leifson's*

A straight inoculating wire with suspension of each isolate was inserted into tube containing Hugh and Leiston's agar medium. Paraffin oil was over layered in one tube for observation of fermentative mode of utilization on of sugar; the tubes were then incubating at 37 °C for 24 to 48 hrs. After incubating color changes from bluish green to yellow was taken as positive test.

II) *Sugar fermentation test*

Tests were carried out by inoculating a loopful suspension of each isolated in to sterile Norris (1965) BASAL medium containing 1% solution of carbohydrates such as glucose, mannitol, lactose, sucrose, xylose, galactose with bromothymol blue (BTB) as an indicator and inverted Durham 's tube. Tubes were incubated at 37 °C for 24 hr. Acid production was detected by seeing development of yellow color and gas production by accumulation in Durham's tube.

III) *Lecithinase test*

A loopful culture of each isolate was spot inoculated on sterile lecithenase agar and plates were incubated at room

temperature for 24 hours. activity of lipase was detected by observing zone of clearance around the colonies.

IV) *Catalase test*

Growth of each isolate from nutrient agar plate were picked with sterile nichrome wire loop and dipped into 10% H_2O_2.evolution of gas was observed as positive reaction.

V) *Oxidase test*

Growth of each isolate was transferred by sterile glass rod on to Whatman filter paper strip moistened with freshly prepared 1% aqueous solution of oxidase reagent (N,N,N',N 'tetramethyl –Paraphenyldiaminedihydro chloride) and observed for appearance of purple colour on strip within 10 seconds.

VI) Arginine hydrolysis test

Each isolate was inoculated in sterile tube of arginine broth and incubated at ambient temperature for 48 hrs. The ability of isolates to hydrolyze the arginine was detected by addition of Nesseler's reagent.

Synthesis of silver nanoparticle (AgNPS)

The loopful sample of all given isolates were streaked on agar nutrient medium and incubated at 37 °C. for 24 hr. Then select isolated a same colony appearance and prepare to inoculated in 250 ml Erlenmeyer flask with 100 ml nutrient broth. Then the flacks were incubated at 24-48 hrs. at 37 °C temperature. After that the medium which is in flacks are centrifuged by 10000rpm. / 10min. Then collect supernatant in another Erlenmeyer flask and this supernatant was studied for extra cellular silver nanoparticles production. For that it mix with 1mMand2mM $AgNO_3$ soln. at the same time the pellet is used as synthesis of intra cellular Silver nanoparticles. In that pellet add 1mMand2mM $AgNO_3$ solution and detect intracellular nanoparticles. It is detected by visual observation in extracellular as brown colour in the given flask containing solution.

Characterization of silver nanoparticles

It is characterized by using nanoparticles tracking analysis. And also X-Ray Diffraction measurement.

Nanoparticles tracking analysis

The sizes of nanoparticles which are synthesized in broth are detected by using nanoparticles tracking analysis.

X-RAY Diffraction (XRD) measurement

For XRD measurements centrifuged pellet is used for X-Ray diffraction measurements.

Antimicrobial activity

The nanoparticles are synthesized in broth is used in antibacterial activity for that use various pathogenic bacteria and fungal those bacteria include *E. coli, Bacillus, Pseudomonas, Klebsiella pneumoniae, Staphylococcus aureus, Salmonella typhi.* The fungus used for that is *Aspergillus spps.* while the yeast includes *Candida albicans.*

RESULTS AND DISCUSSION

Collection of samples

The sample from jewelry metal and molded washed waste. It was from Sangli and Kolhapur (Hupari) region. The temperature of that region when the collection of samples was 37 °C. The collected samples were 5. Out of that 3 are in Hupari and 2 are in Sangli region.

Isolation of silver nanoparticles synthesizing bacteria

About 5 samples we were isolated a strain. Out of the 5 strains were isolated on 2 mM (2mM $AgNO_3$ + NA) and 4 strains were isolated on 1mM (1mM $AgNO_3$ + NA). The isolates are coded as (Table 1).

Table 1 Coding of isolate

S. No.	Isolate from1mM	Isolate from 2mM
1.	SPS1	SPS2
2.	SPL1	SPL2
3.	HPS1	HPS2
4.	HWP1	HWP2
5.	-	HMS2

Characteristics of isolates

a) *Colony characters*

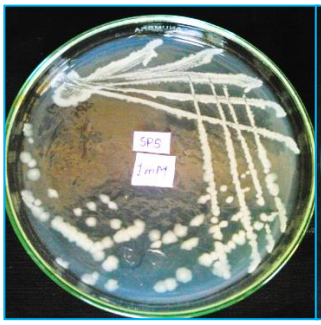
The isolates were obtained when the samples streak on nutrient agar and incubated at 37 °C. for 24-48 hrs. The results were obtained from those plates in the studies are presented in (Table 2, Photograph 1-9].

b) *Morphological character*

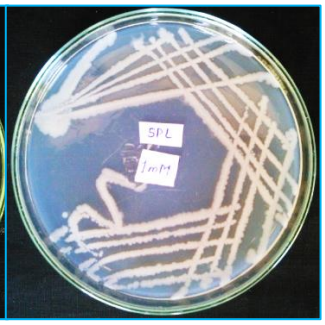
All the isolates appeared Gram positive rod, except 2mm containing sample as SPS2 (As per table 4.3). SPS1, HPS1, HWP1 in 1mm concentration and SPS2, SPL2 in 2mm concentration all these isolates are motile. While remaining that is SPL1 and HPS2, HWP2, HMS2 are non- motile in 1mmand 2mm respectively (Table 2).

Table 2 Colony characteristics of isolates

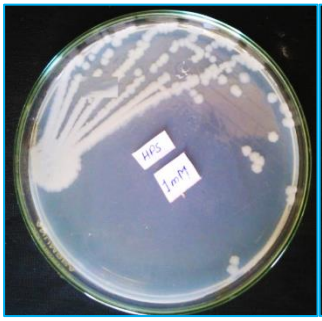
Concentration	Isolates	Colony Characters						
		Size (mm)	Shape	Colour	Margin	Elevation	Opacity	Consistency
1mM	SPS1	3mm	Circular	White	Regular	Flat	Opaque	Moist
	SPL1	2mm	Circular	White	Irregular	Flat	Opaque	Moist
	HPS1	5mm	Circular	White	Regular	Flat	Opaque	Moist
	HWP1	2mm	Irregular	white	Irregular	Flat	Opaque	Moist
2mM	SPS2	2mm	Circular	White	Irregular	Flat	Opaque	Moist
	SPL2	4mm	Irregular	White	Irregular	Flat	Opaque	Moist
	HPS2	4mm	Circular	White	Irregular	Flat	Opaque	Moist
	HWP2	4mm	Circular	Creamy white	Regular	Flat	Opaque	Moist
	HMS2	3mm	Circular	White	Regular	Flat	Opaque	Moist



Photograph 1 Colonial morphology of isolate No. SPS1onNutrient agar medium



Photograph 2 Colonial morphology of isolate No. SPL1 on Nutrient agar medium



Photograph 3 Colonial morphology of isolate No. HPS1on Nutrient agar medium

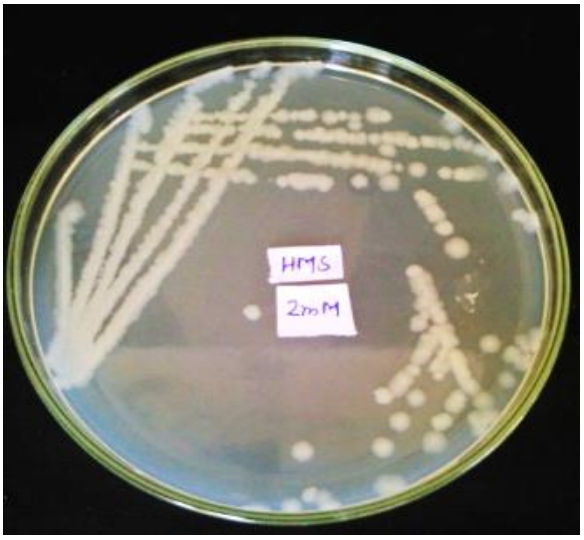
Photograph 4 Colonial morphology of isolate No. HWP1 On Nutrient agar medium

Photograph 5 Colonial morphology of isolate No. SPS2 on nutrient agar medium

Photograph 6 Colonial morphology of isolate No. SPL2 on nutrient agar medium

Photograph 7 Colonial morphology of isolate No. HPS2 On Nutrient agar medium

Photograph 8 Colonial morphology of isolate No. HWP2 on nutrient agar medium



Photograph 9 Colonial morphology of isolate No. HMS_2 On nutrient agar medium

Physical characterization

a) *Temperature requirement*

The results of growth and nanoparticles production of the isolates at various incubation temperature were shown in following (Table 3).

In above table it is seen that the nanoparticles were produced efficiently at 37 °C temp by isolate SPS2 and SPL2 on basis of colour change [36], while remaining isolates showed negligible amount. At 26 °C. similar type of results were seen. At 20 °C isolates SPS2, SPL2, HPS2, HWP2, HMS2 showed negligible amount of nanoparticles production on basis of colour change. At temperature 0 °C similar type of results was obtained. From above results it indicates that at concentration 2mM of $AgNO_3$ treated cells were produces sufficient amount of nanoparticles at temperature 37 °C. While at concentration 1mM of $AgNO_3$ isolates produces negligible amount of nanoparticles at 37 °C, 26 °C, 20 °C, 0 °C.

Table 3 Effect of temperature on the synthesis of silver nanoparticles

Concentration	Isolates	Temperature ranges			
		0°C	20°C	26°C	37°C
1mM	SPS1	-	-	+	+
	SPL1	-	-	+	+
	HPS1	-	-	+	+
	HWP1	-	-	-	-
2mM	SPS2	+	+	++	+++
	SPL2	-	+	++	+++
	HPS2	+	+	+	+
	HWP2	-	+	+	+
	HMS2	-	+	+	+

+ = Growth and nanoparticles production

- = Only growth

Table 4 Effect of salt concentration on synthesis of silver nanoparticle

Concentration	Isolates	Ranges of salt concentration			
		0·5%	1%	1.5%	2%
1mM	SPS1	+	-	+	+
	SPL1	+	-	+	+
	HPS1	+	-	+	+
	HWP1	+	-	-	+
2mM	SPS2	+	+	++	+++
	SPL2	+	+	++	+++
	HPS2	+	+	+	+
	HWP2	+	+	+	+
	HMS2	+	+	+	+

b) *Salt concentration*

The results of growth and nanoparticles production of the isolates at different salt concentration were as shown in (Table 4). From the table it is seen that nanoparticles were production increases efficiently by 2mM $AgNO_3$ treated cells at salts concentration ranging from 0.5% to 2%. While isolates of 1mM $AgNO_3$ treated cells showed negligible amount of nanoparticles production at all range of salt concentration (Table 4).

Table 5 Observation table for Hugh and Leifson's test

Concentration	Isolates	Aerobic tube	Anaerobic tube (with sterile liquid paraffin oil layer)
1mM	SPS1	®	®
	SPL1	-	®
	HPS1	®	®
	HWP1	®	®
2mM	SPS2	®	®
	SPL2	®	®
	HPS2	®	®
	HWP2	®	®
	HMS2	-	®

+ = positive, - = negative test, ® = acid and gas

Biochemical characterization

The results of the Biochemical characters of the isolates were noted in table:

a) *Hugh and Leifson's tests*

All the isolates showed fermentation of glucose in tube incubated aerobically as well as in the tube incubated an anaerobically with paraffin cover. This indicated that all the isolates were facultative anaerobic in nature (Table 5).

b) *Sugar fermentation test*

Sugar utilization and fermentation test of all the isolates were performed and the results were listed in the table. From the table it was observed that SPS1, SPL1, HPS1, HWP1 isolates produce only acid by the utilization of Glucose, Fructose, Sucrose, while they do not utilize Maltose, Mannitol and Cellobiose. In the isolates of 2mM concentration containing SPS2, SPL2 utilize all above discussed sugar and produce acid and gas except Cellobiose sugar. HPS2 utilize all above discussed sugar except Mannitol and Galactose.HWP2 only utilize Glucose and Fructose. While HMS2 utilize Fructose, Galactose and Lactose (Table 6).

Table 6 Observation table for sugar fermentation test

Concentration	Isolates	Glucose	Fructose	Sucrose	Maltose	Mannitol	Galactose	Lactose	Cellobiose
1mM	SPS1	+	+	+	-	-	+	+	-
	SPL1	+	+	+	-	-	-	+	-
	HPS1	+	+	+	-	-	-	-	-
	HWP1	+	+	+	-	-	+	-	-
2mM	SPS2	®	®	®	®	®	®	®	-
	SPL2	®	®	®	®	®	®	®	-
	HPS2	+	®	+	+	-	-	+	weak
	HWP2	+	®	-	Weak	Weak	-	-	-
	HMS2	-	+	-	Weak	Weak	®	®	-

+ = only acid, - = negative test, ® = acid and gas

c) *Lipase and Lecithinase test*

When isolated colonies prepare the suspension and spot inoculated on Egg yolk agar after incubation showed zone of clearance. The results were mentioned in given (Table 7).

Table 7 Observation table for lipase and lecithinase test

Concentration	Isolates	Lipase	Lecithinase
1mM	SPS1	-	-
	SPL1	-	-
	HPS1	-	-
	HWP1	-	-
2mM	SPS2	-	-
	SPL2	-	-
	HPS2	-	-
	HWP2	-	-
	HMS2	-	-

+ = positive, - = negative

Table 8 Observation table for casein hydrolysate test

Concentration	Isolates	Casein hydrolysate test
1mM	SPS1	+
	SPL1	+
	HPS1	+
	HWP1	+
2mM	SPS2	+
	SPL2	+
	HPS2	+
	HWP2	+
	HMS2	+

d) *Casein hydrolysate test*

When the prepared suspension containing loopful was inoculated on milk agar and observes the zone of hydrolysate. The results are mentioned in the (Table 8). The isolate showed clear hydrolyzed zone surrounding the growth indicate positive test.

Table 9 Observation table for catalase test

Concentration	Isolates	Catalase test
1mM	SPS1	+
	SPL1	+
	HPS1	+
	HWP1	+
2mM	SPS2	+
	SPL2	+
	HPS2	+
	HWP2	+
	HMS2	+

e) *Catalase test*

When the growth was dipped in 10%H_2O_2 evolution of gas was observed indicated as positive test (Table 9).

Table 10 Observation table for oxidase test

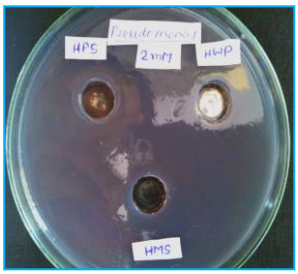
Concentration	Isolates	Oxidase test
1mM	SPS1	+
	SPL1	-
	HPS1	-
	HWP1	-
2mM	SPS2	-
	SPL2	-
	HPS2	-
	HWP2	-
	HMS2	-

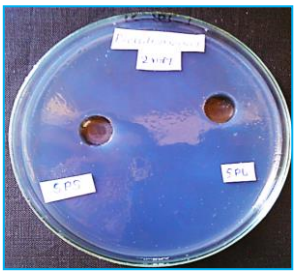
f) *Oxidase test*

When the growth of isolates on were rubbed on filter which are moistened with oxidase reagent. If purple colour showed in 10min indicates positive tests. The only 1mM containing SPS1 isolates showed positive test remaining test isolates showed negative test (Table 10).

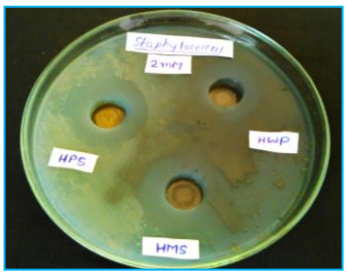
Photograph 10 Flasks containing silver nanoparticle

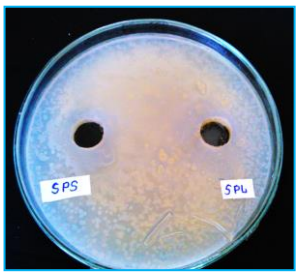
h) *Antibacterial and antifungal activity of silver nanoparticles*



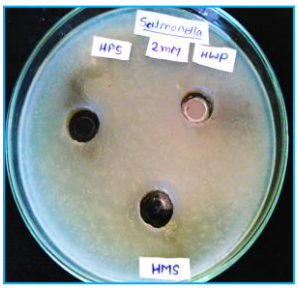


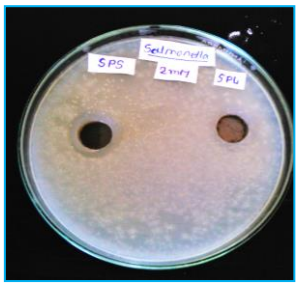
Photograph 11 Antibacterial activity against *Pseudomonas aeruginosa*



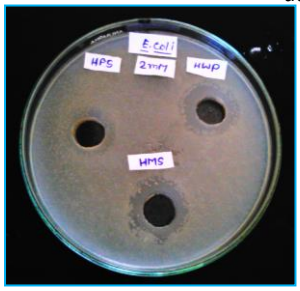


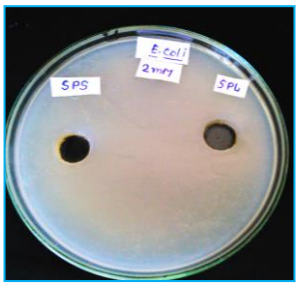
Photograph 12 Antibacterial activity against *Staphylococcus aureus*



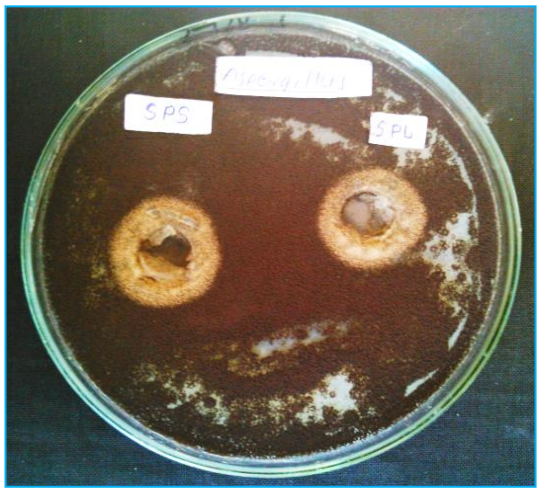


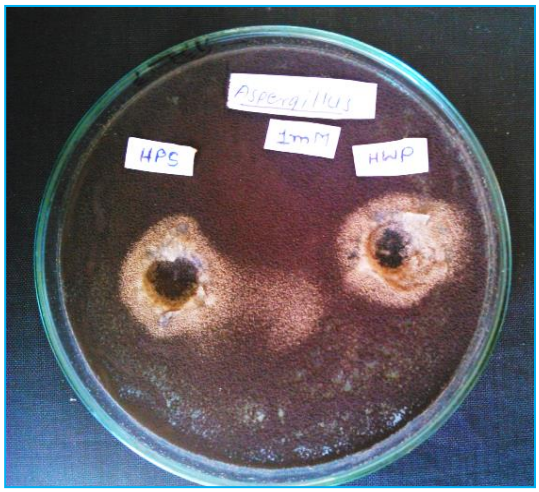
Photograph 13 Antibacterial activity against *Salmonella typhi*



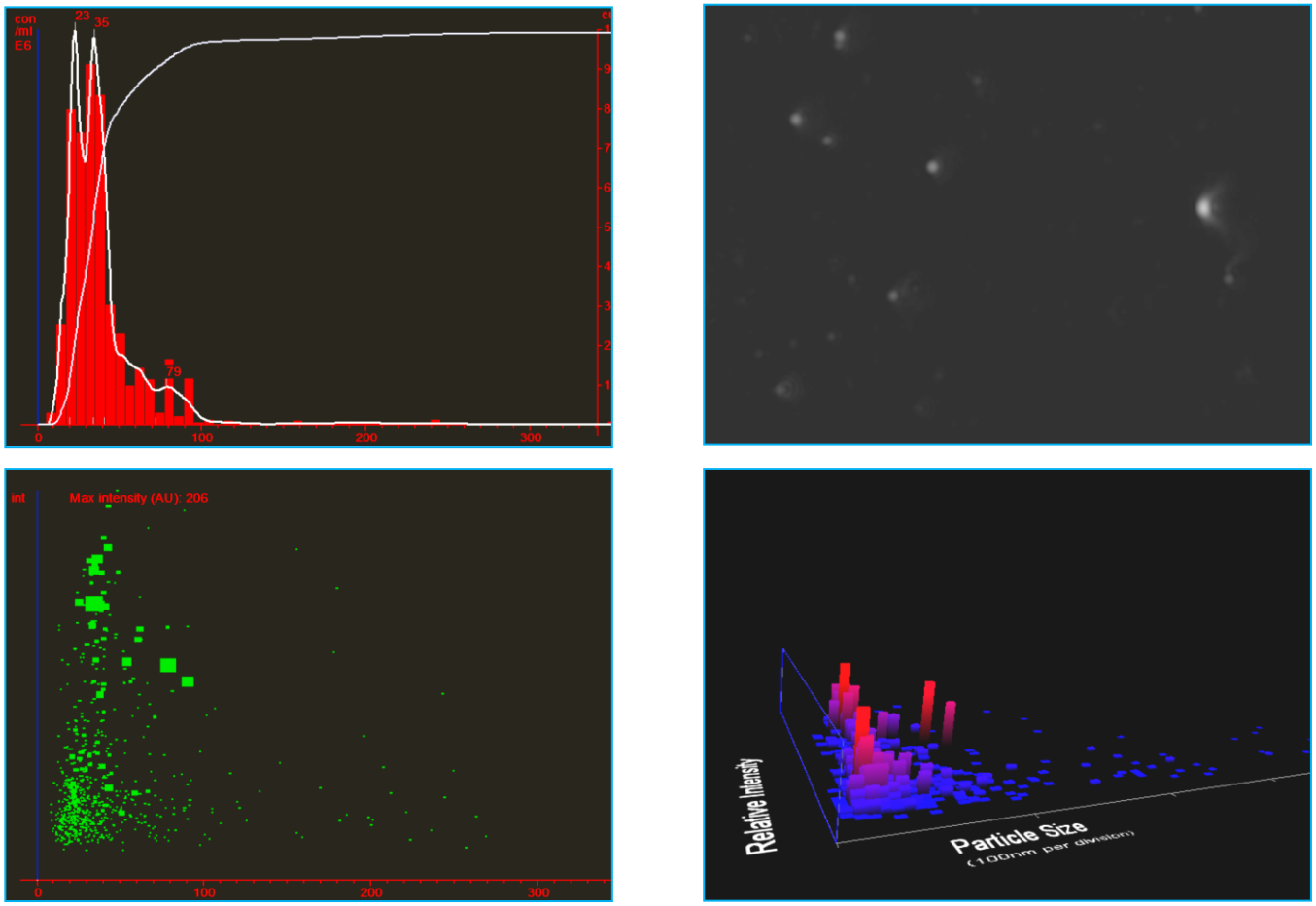


Photograph 14 Antibacterial activity against *E. coli*

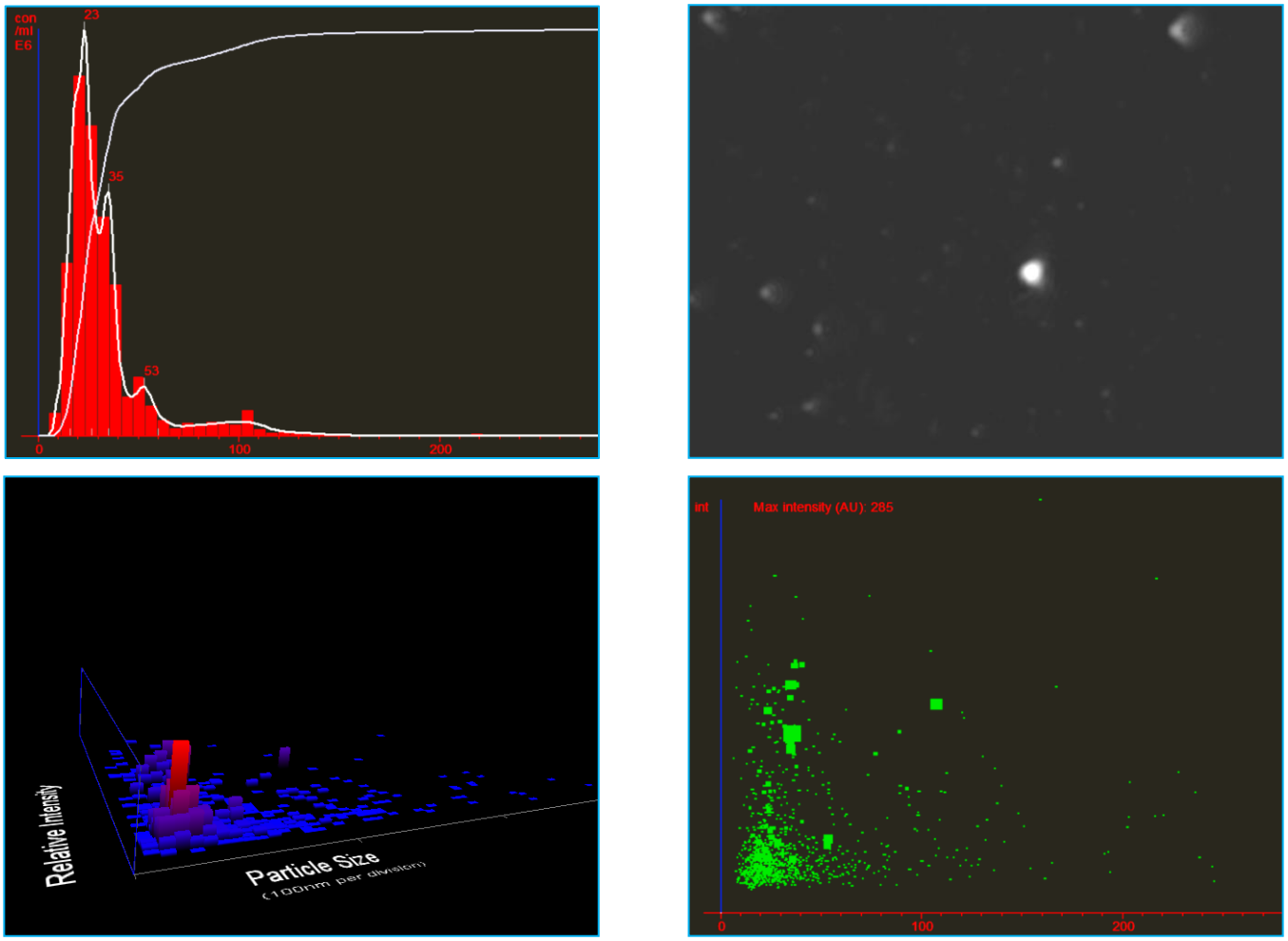




Photograph 15 Antifungal activity against *Aspergillus spps*



(i) Image 3 Images for nano particles detection by using nanoparticle tracking analysis. Sample: 1(SPL2)$^{+}$



Images for nano particles detection by using nanoparticle tracking analysis. Image Sample No 4: Sample No. 2 (HPS2)

Particle size was determined by nanopartical size counter and results represented as 3D graph.The size distribution of sample no 1: (SPS2) was mean=45nm, mode=23nm, SD=48nm while in sample no 2 (HPS2) was mean= 36nm, mode=23nm, and SD=31nm.

j) *XRD of silver nanoparticle (sample SPS2)*

The results of XR D of our sample was compared to standard result and it was confirmed that the nanoparticles are formed on the basis of peaks at 2Θ value is near about 38.0°. The organisms were isolated from Jewelry molded waste and used for silver nanoparticle synthesis. The all isolated which was synthesizing silver nanoparticles were studied thoroughly. Synthesized silver nanoparticles were characterized by using nanopartical tracking analysis and X- ray diffraction measurements. The effect of various parameters in the synthesis of silver nanoparticles with respect to temperature, salt concentration stability was evaluated. The isolates in 1mM showed temperature stability SPS1 in between 20 °C to 37 °C while SPL1, HPS 1, HWP1, were found to more efficient at 37 °C. The isolates in 2m M $AgNO_3$ concentration showed temperature optima in between 10 °C to 37 °C. In case of salt concentration effect 0.5 to 2.0 % salt concentration was used. The salt was added when the growth was observed in the form of turbidity followed by addition of 1mM and 2mM $AgNO_3$ solution and incubated at 37 °C for 24 hrs.

The brown-coloured broths were observed which indicates the formation of silver nanoparticles. The synthesized silver nanoparticles were characterized after extraction; using suitable techniques. The XRD pattern compared with earlier reports to confirm the silver nanoparticles. The particle size was calculated using nanoparticles size counter. From all the analytical studies it was confirmed that isolated microbes synthesized silver nanoparticles in significant amount. The nanoparticles are further exploited for its antimicrobial activity which was significantly higher. Overall, from the present studies it can be concluded isolated microbes successfully synthesizes the silver nanoparticles, which can be used for variety of applications including environmental as well as medicinal and industrial.

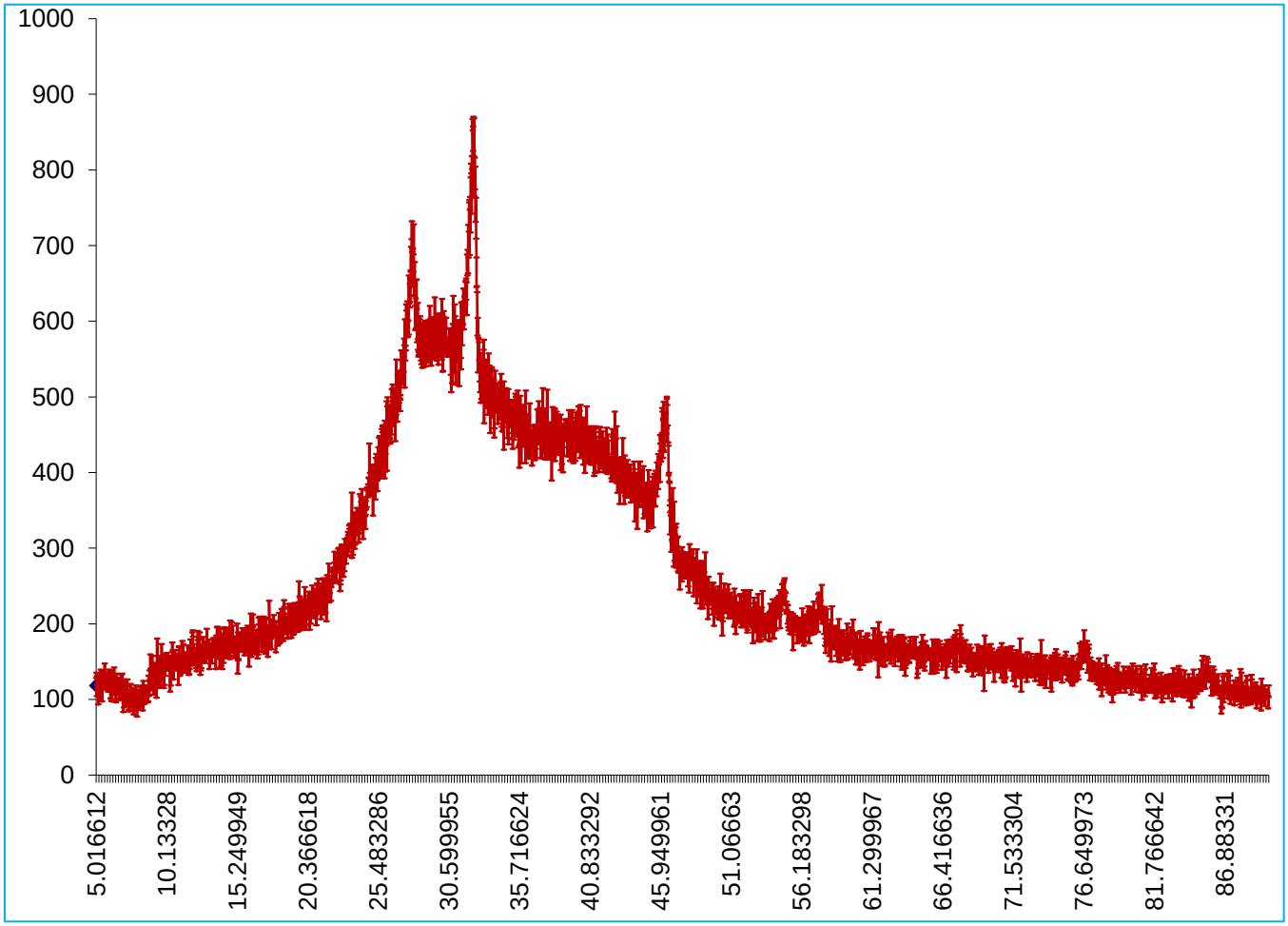


Image 5 XRD image of silver nanoparticles (sample SPS2)

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