Using of Silver Nanotechnology Lactobacillus Bacterial Synthesis Isolates from Teeth Root Canal as Antibacterial Infection

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Abstract

Silver nanoparticles were made using cell supernatants from Lactobacillus species. Measurements were made of the optical density of AgNPs produced by completely distinct cell supernatants. Because Lactobacillus demonstrated the best AgNP production, it was selected. Before medium optimization, liquid Ag+ ions were converted to AgNPs once again and added to the eubacterial cell-free supernatant during ten hours of incubation. The nitrate enzyme protein and other reductases may have contributed to the creation of silver nanoparticles. During incubation, a color shift from light yellow to brown was seen, whereas the management had no color change. At about 430 nm, spectrophotometric examination of Ag NPs yields a distinctive Surface Plasmon Resonance band, confirming the existence of spherical or approximately spherical atomic number 47 nanoparticles. The formation of silver nanoparticles was found to be simpler in the cell free supernatant that had been exposed to electromagnetic radiation after mixing with AgNO3 solution than it had been in the cell supernatant that had been exposed to electromagnetic radiation prior to mixing with AgNO3 solution. During this study, the antimicrobial activity of the synthesized AgNPs against different species of extremely infective multidrug resistant lactobacillus was investigated, and AgNPs was compared to antibacterial medicine as Tetracycline. The synthesized AgNPs were tried to own antimicrobial activity against all the tested microorganisms. The diameters of microorganism inhibition zones (mm) measured for lactobacillus was 27.67 ± 0. 577.Aim to studyThe aim of this study to mistreatment of using of lactobacillus microorganism synthesis isolates from teeth passageway from silver technology as medicinal drug infection. The antimicrobial activity was assessed by mensuration the diameter of inhibition zones by crucial the minimum repressing concentration (MIC) and by determining the minimum fatal concentration (MLC).

Keywords: Nanotechnology, silver, antibacterial infection, *Lactobacillus*.

Introduction

Growing emphasis has been paid to the synthesis of nanoparticles as a key area of the interface between applied science and biotechnology as a result of a growing need to create environmentally friendly materials synthesis methods (Rahimi et al., 2015). With the current interest in research due to the growing microbic resistance against metal ions and antibiotics and the resulting development of resistant strains, auriferous nanoparticles are the most promising because they exhibit good medicinal properties because of their enormous expanse to volume ratio (Hamouda, 2012). Because silver has long been known to be very harmful to 116 different types of microorganisms, silver-based compounds are often utilized in disinfection applications (Singh et al., 2008). Commercially, several silver salts and their derivatives are used as antibacterial agents. Silver ions have a very well-known bactericidal effect on bacteria, however the bactericidal process is still partially understood. It has been hypothesized that important enzymes' thiol teams interact with ionic silver in a potent manner to inactivate them (El-Batal et al., 2013). Deoxyribonucleic acid seems to lose its capacity to replicate after the microorganisms have been exposed to silver ions, according to experimental evidence. Due to the development of microscopic silver and sulfur-shaped electron-dense granules, several investigations have found indications of structural alterations in the plasma membrane (Yang et al., 2009). The science and engineering involved in the design, synthesis, characterisation, and application of materials and technologies whose lowest practical organization in at least one dimension is on the metric linear unit scale shall be defined as applied science (one-billionth of a meter). The diverse scientific subject of nanotechnology has expanded rapidly in recent years and is experiencing rapid growth (Silva, 2004). As a consequence of nanoscience's and nanotechnology's enormous potential to advance fields as diverse as medicine research, water purification, information and communication technologies, and the creation

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of stronger, lighter materials, it will undoubtedly be beneficial for human health care. Large health advantages will surely result from applied science research on human health care. The promise of transformative developments in genomics, AI, communications, and medicine will be modeled after the origins of nanotechnology (Sahoo et al., 2007). Although there are far too many and varied possible uses for nanotechnology to discuss in detail, one of its greatest benefits will undoubtedly be the creation of cutting-edge and powerful medicinal therapies (Emerich & Thanos, 2003). The physical characteristics of metal particles in the metric linear unit size range are completely different from those of the bulk material. Due to their morphologies, which have exceptionally active sides, they are able to display extraordinary qualities that increase chemical process activity (Zhang et al., 2017). Through the reduction of the metal ions, microorganisms like bacteria and fungi today play a significant role in the treatment of hazardous metals. Because silver has long been known to be very poisonous to a wide variety of 116 microorganisms (Tabak et al., 2005), silver-based compounds are widely employed in numerous disinfection applications (Vasilev et al., 2009). Commercially, several silver salts and their derivatives are used as antibacterial agents. Silver ions have a very well-known bactericidal effect on bacteria, however the bactericidal process is still partially understood (El-Batal et al., 2013). It has been hypothesized that ionic silver interacts with important enzymes' thiol teams strongly and renders them inactive. Although the usefulness and efficacy of silver ions in bactericidal applications is undeniable, the unique features of nanoparticles allow for the creation of new bactericides at a lower cost (Morones et al., 2005). Metal particles of varying sizes, measured in metric linear units, have physical characteristics that are entirely different from those of the bulk material (Koziej et al., 2014). Due to their morphologies, which have exceptionally active sides, they are able to display extraordinary qualities that increase chemical process activity (Singh et al., 2008).

Materials and Methods

Patients and collection of samples:

The research was conducted from March (2021) to July for a total of five months (2021). 100 individuals with severe inflammation in their teeth's root canals visited a dental facility in Hilla city. Disposable cotton swabs were used to capture the sample from each case's abscess at the location of the inflammation, and it was then examined under a microscope and the bacteria were isolated according to protocol. Samples were carefully gathered to prevent contamination. The remaining sample was sent to the Department of Microbiology for further research after which it was plated onto Blood Agar and Nutrient Agar media and incubated at (37 oC) for (24) hours. Gram stain, colony morphology, biochemical tests, and the Vitek 2 technique for identifying Lactobacillus spp. were used to diagnosis the bacteria that were isolated (Hessan & Jassam, 2021).

Ethical Approval

Each patient gave their valid permission before being included in the trial.

Identification of bacterial isolates by gram stain, biochemical tests

Each isolate underwent identification tests including cultural, morphological, and biochemical traits in accordance with (Baron et al., 1994, McFadden, 2000).

Identification of Streptococcus anginosus isolates with Compact VITEK-2 System

Utilizing the Compact VITEK-2 System, all Lactobacillus spp. isolates were screened and identified (BioMerieux). This form of identification is phenotypic and relies on biochemical processes to identify the isolates. There are 64 wells on the Vitek-2 card, each holding a separate fluorescent biochemical experiment. Phosphatase, urea, nitrate, and actidione assays were performed on 20 of the 64 samples for carbohydrate assimilation. Automatically filling, sealing, and transporting the cards into the associated incubator (35°C) were all handled by the Vitek-2 machine. According to a certain algorithmic framework, each output report is decoded. According to the ID-GP (identification of Gram-negative bacteria) databank, the collected findings were classified. The corresponding supporting software automatically suggests the ID results from these systems. Only when "poor discrimination" or "no ID" was indicated by the first findings were the tests repeated, and the repeat result was considered for data analysis. On anaerobic culture medium, each strain was inoculated, followed by an overnight incubation at 37°C. According to the manufacturer's instructions, the phenotypic VITEK-2 Systems approach was utilized to identify a single isolated colony (BioMerieux).

Irradiation source

At NCRRT, the irradiation procedure was allocated. At the time of the tests, radiations were given using a gamma-irradiation system that used a Co-60 source (Gamma cell 4000-A-India) at a rate of 3.31 kGy/hr.

Production of cell supernatant from different Lactobacillus species

Distinct isolates were grown aerobically in nitrate medium to produce the cell supernatant for the manufacture of silver nanoparicles from entirely different

genuine bacteria species. The culture flasks were shaken at a rate of 120 while being incubated. After twenty-four hours, the cell supernatants were collected by activity at 6,000 rpm for ten minutes at 6 °C.

Synthesis of silver nanoparticles

With a little modification, the process described by (Boroumand et al., 2015) was used to synthesize silver nanoparticles. Silver nanoparticles were created using the previously stated cell supernatant. AgNO3 was added to erlenmeyer flasks holding 100 ml of optimal medium's cell supernatant at a concentration of 1 mM for five minutes. A UV-visible spectrophotometer with a 1 nm resolution was used to record the sample's absorption spectra. In a typical experiment, the cell supernatant was subjected to doses of 0.25 to 3 kGy of gamma radiation at ambient temperature once before and once after mixing with 1 Mm silver nitrate.

Screening of the most potent local *Lactobacillus* isolates for microbiological synthesis of silver nanoparticles

Silver nanoparticles were created using cell supernatants from several lactobacillus species that had been cultured on nitrate medium. We investigated the optical density of AgNPs produced by various cell supernatants (Dakhil, 2017).

Estimation of nitrate reductase activity

On the basis of a modified approach, the nitrate reductase activity was (Simmons & Moss, 1978). The resulting cell-free supernatant served as the crude enzyme. By mixing 25 metric linear units of metal phosphate buffer with 30 metric linear units of nitrogen and 0.05 metric linear units of ethylenediaminetetracetic acid at a hydrogen ion concentration of 7.3, the protein substrate answer was prepared. A 100 l aliquot of enzyme was added to 1.8 cc of substrate solution, and 100 l of 2.5 metric linear unit -nicotinamide purine dinucleotide reduced kind solution (-NADH) was added to promote the reaction. For five minutes, the reaction mixture was incubated at 30 °C. The reaction was then immediately stopped by adding and transferring 1 ml of a 58% sulphanilamide solution in 3M HCl. N-(1-naphthyl) ethylenediamine dihydrochloride solution (NED), 0.77 mM, was then added and swirled to combine. After incubating the mixture for five minutes, color development was seen. At 540 nm, the absorbance was measured. The definition of a unit is a micromole of group produced every minute.

Characterization of silver nanoparticles

Before and after mixing with the AgNO3 solution, cell supernatant was subjected to gamma radiation at dosages of 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, and 3.00 kGy at room temperature. After being exposed to radiation, the created AgNPs were examined by UV-vis, DLS, TEM, and FT-IR spectroscopy.

UV/vis Spectral Analysis

AgNPs' UV/VIS spectra were captured utilizing various wavelengths. A UV/vis photometer that worked between 200 and 900 nm with a resolution of one nm.

Transmission Electron Microscopy (TEM)

Utilizing TEM, the size and shape of the produced nanoparticles were documented. In order to prepare TEM investigations, carbon-coated TEM grids were drop-coated with silver nanoparticles. After allowing the film on the TEM grids to dry, the excess solution was eliminated using blotting paper.

Determination of zone of inhibition

Using the agar well diffusion technique, the synthesized AgNPs were evaluated for their antibacterial efficacy against Lactobacillus isolated from clinical samples (Garibo et al., 2020). At the National Cancer Institute, it was verified using the MicroScan WalkAway-96 SI System (Dade Behring, Germany). Using sterile cotton swabs, standardized suspensions of each tested strain (108 cfu/ml for bacteria and 106 cfu/ml for fungi) were swabbed evenly onto sterile Muller-Hinton Agar (MHA) (Oxoid) plates. Using gel puncture, 10 mm-diameter wells were punched into the agar medium. Each well received 100 ul of a silver nanoparticle solution (200ppm) using a micropipette. Zones of inhibition were evaluated 24 hours after incubation at 37°C. Positive tests for antimicrobial activity were made using the antibacterial drug tetracycline. The results were reported as mean values SD after the determinations were made in triplicate (NCIS, 2019).

Determination of minimum inhibitory concentration (MIC)

The MICs were calculated using repeated two-fold dilutions of AgNPs in concentrations ranging from 1600-0.049 ppm in Luria Bertani (LB) broth in triplicate along with a positive control tube (the microorganism in LB broth) and a negative control tube (LB broth) (CLSI, 2019). After 24 hours of incubation at 37 °C with starting inoculums of 0.1 OD at 600 nm, the MIC was calculated. The MIC is the lowest AgNP concentration at which 99% of the tested microorganism's growth is fully visibly inhibited. The concentration of AgNPs (MIC50 and MIC90, respectively) that may prevent 50% and 90% of a population of microorganisms from growing visibly was also established. After MICs were established, 50 ul aliquots from all tubes in which no discernible growth was seen were seeded onto MHA plates without the addition of AgNPs and cultured for 24 hours at 37 °C. The MLC, which completely eradicates the original microbial population, was defined as the lowest concentration of AgNPs with no growth on the MHA medium (). The MIC and MLC were used to reflect the bactericidal and bacteriostatic activity of the AgNPs against the tested bacterial strains (MBC & MFC for bacteria

strains). All of the examined strains were subjected to an antimicrobial analysis of the synthesized AgNPs, which included zone of inhibition, MIC, MIC50, MIC90, and MLC values, both before and after their in vitro exposure to a dosage level of 24.41 Gy gamma radiation. This was done in order to research the antibacterial activity of AgNPs against microorganisms that cause infections in patients receiving radiation.

Statistical analysis

In order to do the statistical analysis, SPSS version 23 was used. The format for continuous variables was (Means± SD). The means of the two groups were compared using a student t-test.

Results

In this research, 100 patients with acute inflammation in their teeth's root canals visited a dental clinic in Hilla City. The samples were grown aerobically and anaerobically at (37 oC) for (18–24) hours. Out of 100 specimens, only 22 (22%%) isolates were connected to lactobacillus, while 78 (78%) related to other kinds of microorganisms, as indicated in the table. Identification of Lactobacillus spp. was dependent on colony morphology, microscopically, and biochemical tests as initial identification (Table1).

Table (1): Identification of *Lactobacillus spp.* depended on the colonial morphology, microscopically, and biochemical tests and specific positive cards of Vitek 2 system

Total No. of samples	Initial identification of *Lactobacillus spp.*	Other microorganisms	Identification of *Lactobacillus spp.* by Vitek 2 system
100	22(22%)	78(78%)	22(22%)

The ability of Lactobacillus to produce silver nanoparticles was examined. The maximum optical density and best results were produced by the Lactobacillus strain (2.7 absorbance). Within 10 hours of being introduced to Lactobacillus' cell-free supernatant, aqueous Ag+ ions were transformed into AgNPs. Prior to media optimization, after incubation. A color shift from yellowish yellow to brown was seen throughout incubation, whereas the control exhibited no color change. By using spectrophotometric analysis, a typical SPR band for AgNPs was found at around 430 nm, confirming the presence of spherical or nearly spherical Ag nanoparticles. At a concentration of 1 mM silver nitrate and 5 ml of cell-free supernatant, the UV-Vis spectra absorbance as a function of time shows that the reaction was complete during the first 12 hours and that an increase in time has no effect on the creation of AgNPs, as shown in Figure (1).

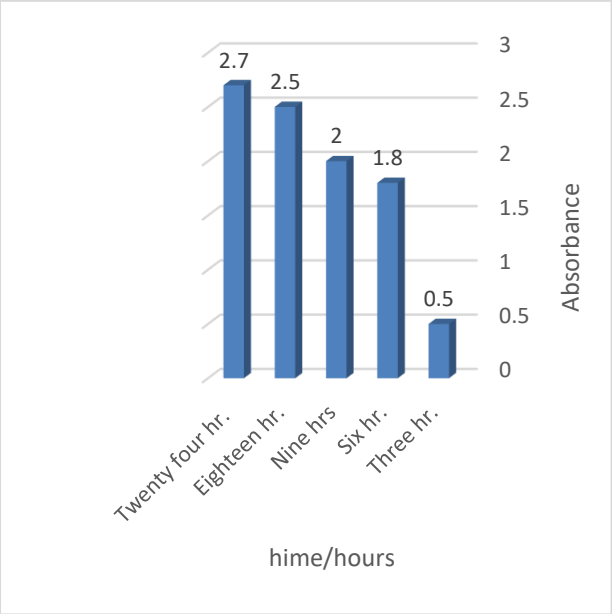

Figure (1): Effect of time on formation of AgNPs

Silver nanoparticle dispersions exhibit vibrant hues as a result of Plasmon resonance absorption. As a result, metallic nanoparticles exhibit a distinctive optical absorption spectrum in the UV-visible range, as seen in Figure (2). AgNPs had a high, wide peak in their UV-visible spectra, which was positioned between 420 and 440 nm.

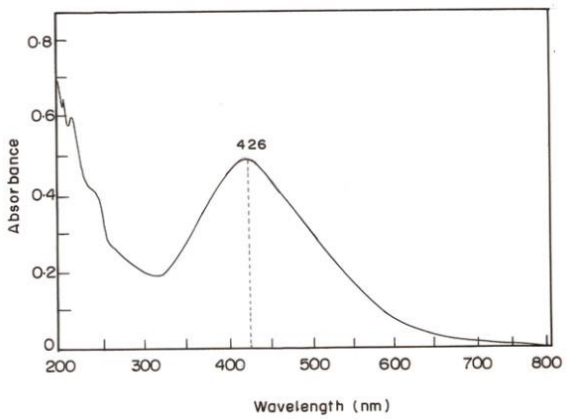

Figure (2): UV VIS-Spectrum of silver nanoparticles

When bacterial cell free supernatant was exposed to various gamma radiation doses before and after being mixed with silver nitrate solution, it was discovered that the process of producing silver nanoparticles was more effective when the cell free supernatant was exposed to radiation after being mixed with AgNO3 solution (Figure 3). Figure 4 illustrates the production of silver nanoparticles with a peak appearing at 430 nm as a measure of the rise in the synthesis of these particles.

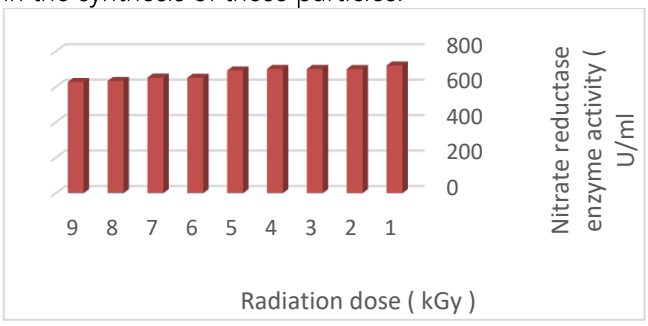

Figure (3): Effect of different doses of gamma radiation on nitrate reductase enzyme activity Radiation dose: 1: control, 2: 0.25, 3: 0.5, 4: 0.75, 5:1, 6:1.25, 7:1.5, 8:1.75, 9: 2

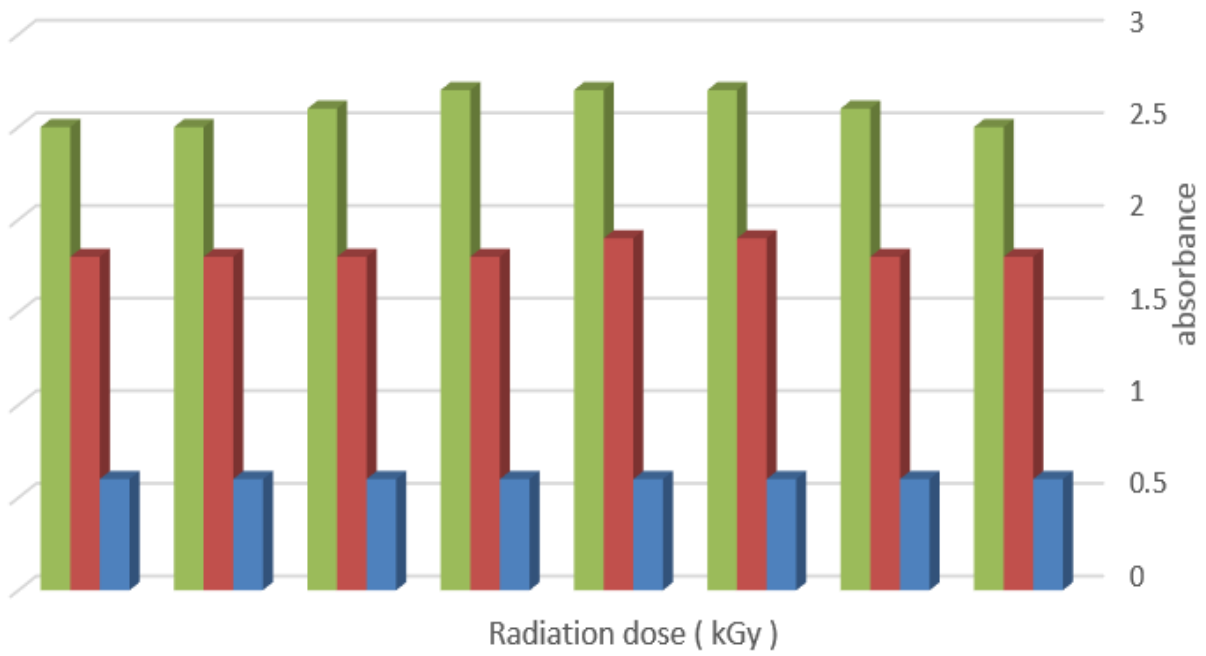

Figure (4): Effect of different doses of gamma radiation on formation of silver nanoparticles
Radiation dose: 1: 0.25, 2: 0.5, 3: 0.75, 4:1, 5:1.25, 6:1.5, 7:1.75, 8: 2, 9:3

The antibiotic activity of produced AgNPs was examined in this work against 22 Lactobacillus species of highly pathogenic, multidrug resistant bacteria. AgNPs' antimicrobial activity was compared to that of antibacterial medicines like tetracycline. Table demonstrates the antibacterial activity of the produced AgNPs against all of the tested pathogens (2). For Lactobacillus, the widths of the bacterial inhibition zones (mm) were measured to be 27.67 0.577. The MIC, MIC50, MIC90, and MLC values of the AgNPs for the chosen strains were determined using the conventional broth macro dilution technique (Table 3). The MIC values of the produced AgNPs against the evaluated clinical isolates demonstrated a significant antibacterial activity; (200 ppm) for Lactobacillus. Before and after in vitro gamma irradiation, AgNPs specifically suppress Lactobacillus growth.

Table (2): The antimicrobial activity (inhibition zone in mm) of the AgNPs synthesized against different strains before and after in – vitro gamma irradiation

strain	Tetracycline (Standard antibacterial agent)	Diameter of inhibition zone. (mm) produced by AgNPs		P-value
		Before	After	
Lactobacillus	26	27.67± 0.577	22.67±1.155	0.0131

Table (3): Minimum inhibitory and minimum lethal concentration values of the AgNPs synthesized for the selected strains

Tested strains	MIC (µg/ml)		MIC 50 (µg/ ml)		MIC 90 (µg/ ml)		MLC (µg/ml)	
	Before gamma-irradiation	After gamma-irradiation	Before gamma-irradiation	After gamma-irradiation	Before gamma-irradiation	After gamma-irradiation	Before gamma-irradiation	After gamma-irradiation
Lactobacillus	200	200	50	50	100	100	400	400

To determine the morphology and form of nanoparticles, TEM measurements were employed. Low-magnification images taken with a TEM The particles are spherical in shape, evenly scattered

(mono dispersed), and lack considerable aggregation, as shown by Figure (5).

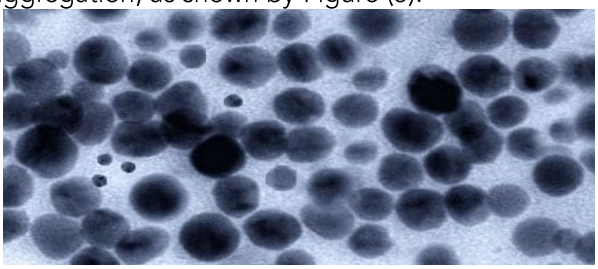

Figure (5): TEM image for silver nanoparticles

Discussion

Because silver has long been known to be very poisonous to 116 different microorganisms (Singh et al., 2008), silver-based compounds are widely employed in a variety of antiseptic applications. 10 Lactobacillus spp. isolates were tested, and the majority of the isolates were found to be positive when the reaction mixture's color changed from yellow to brown, suggesting the assembly of silver nanoparticles (Ag+ to Ag0) (Mohd Yusof et al., 2020). The looks of brown color in AgNO3 treated flask is attributed to the surface Plasmon resonance (SPR) instructed the formation of AgNPs (Manivasagan *et al.*, 2013). It is rumored that reduction of Ag+ to Ag0 happens through enzyme catalysts together with nitrate reductase and alternative proteins. These enzymes discharged within the answer will cut back the caustic to silver nanoparticles through capping agents love proteins. Brown colored precipitate was more used for characterization of silver nanoparticles (Ranganath *et al.*, 2012). The nitrate reductase present in the culture filtrate of Lactobacillus may be the enzyme involved in the creation of nanoparticles. This enzyme transforms silver ions into aluminous silver and is iatrogenic for nitrate ions (Rajesh et al., 2015). AgNPs' biological synthesis might be a hot and eco-friendly alternative approach for manufacturing large amounts. Additionally, microorganisms are easy to handle, capable of genetic manipulation, and do not pose a significant threat (Sharma et al., 2010). A microorganism system may seem like a great option for the synthesis of AgNPs in light of these benefits. Predominant factors including pH, temperature, substrate concentration, and substrate exposure time may be used to change the rate of particle production and, therefore, the dimensions of the nanoparticles. Consequently, it was crucial to maximize the catalyst production (Vaidyanathan et al., 2010). The creation of a replacement method for the synthesis of nanomaterials across a variety of chemical compositions, forms, as well as their separation, is the most significant benefit of this protocol-supported enzyme. The absorbance associate degree analysis qualitative analysis may be a quick thanks to acquire information of silver nanoparticle solution. However, these data alone doesn't tell abundant concerning the silver nanoparticles. A correlation with an optical model should be created (Kumar *et al.*, 2007). According to the silver nanoparticles' spectrum, which was shown, the nanoparticles were assembled everywhere there was a plasmon optical phenomena at 430 nm, which is a hallmark of silver nanoparticles. With unbound electrons inside the physical phenomenon band and charged nuclei, a metal's surface resembles plasma (Anandalakshmi et al., 2016). A collective excitation of electrons within the physical phenomenon band at the surface of the nanoparticles may result in surface Plasmon resonance. The spectra of the silver nanoparticles showed that the nanoparticles had gathered in areas where a plasmon optical phenomena at about 430 nm was present (Kumar & Mamidyala, 2011). With unbound electrons in the conduction band and positively charged nuclei, the surface of a metal resembles plasma. Near the surface of the nanoparticles, surface Plasmon resonance—a collective excitation of the electrons in the conduction band—occurs (Desai et al., 2012). The impact of electromagnetic wave on synthesis of silver nanoparticles showed that it had been simpler once applying to culture supernatant once mix with caustic answer than if applied to culture supernatant before mixing with silver nitrate solution which may be attributed to it just in case of samples irradiated before mixing there may be just one mechanism that is probably the effect of enzyme catalyst, whereas in case of samples irradiated after mixing, synthesis might be because of 2 mechanisms which are reductase enzyme because it is almost not affected with radiation at totally different doses, additionally to radiolytic mechanism (Tashi *et al.*, 2016). The formation of AgNPs just in case of radiation may be attributed to the radiolytic reduction that usually involves lysis of binary compound solutions that has Associate in Nursing economical methodology to cut back metal ions (El-Batal *et al.*, 2014). The creation of new pharmaceutical products is very interested in the synthesis of nanosized particles having medicinal qualities, known as "nanoantibiotics." AgNPs' use as an antibacterial agent is very recent, and it has garnered considerable interest (Hiremath et al., 2014). When compared to those suggested by Jyoti et al., the widths of the bacterial inhibition zones (mm) determined for AgNPs against real bacteria indicated better drug activity (2016). When compared to another work by Hallol (2013), who reported MIC and MBC values of AgNPs about 1800 ppm and 2700 ppm, the findings of the antibacterial activity against real bacteria before and after in vitro gamma irradiation were superior. Every organism is subject to the circumscribed effects of AgNPs, which vary from one to another before and after in vitro gamma irradiation. According to the mechanism of AgNPs' inhibitory action on microorganisms, these organisms are rendered inactive as a result of deoxyribonucleic acid losing its capacity for replication and the expression of the ribosomal fractional protein, among other cellular macromolecules and enzymes required for the production of adenosine triphosphate (El-Batal et al., 2013). AgNPs might attach to the surface of

semipermeable membrane perturbing porosity and respiration functions of the cell or by busy with elements of the microbic lepton transport system. AgNPs may function similarly to antimicrobial drugs used to treat microbial infections, which have four different mechanisms of action, including interference with the synthesis of cell walls, inhibition of protein synthesis, interference with the synthesis of macromolecules, and inhibition of metabolic pathways (Herman & Herman, 2014). According to several research, the conductor+ particle's charge is essential for its antibacterial effectiveness because charged nanoparticles are attracted to charged microbes via their semipermeable membranes (Cavassin et al., 2015). Additionally, Ag NPs may enter inside of bacteria in addition to moving with membrane surfaces. AgNPs have a strong propensity to react with sulfur and phosphorus groups, hence deoxyribonucleic acid-loving molecules and sulfur-containing proteins in cell membranes are likely to be the discriminatory sites for AgNPs (Khurana et al., 2014). AgNPs haven't been shown to cause microorganism resistance presently complicating antibiotic medical aid of bacterial infections. this can be presumptively because of the very fact that, in contrast to antibiotics, AgNPs don't exert their medicament effects during a single specific web site however at many levels love bacterial wall, macromolecule synthesis and deoxyribonucleic acid (i.e. attack a broad vary of targets within the organisms), which suggests that, microorganisms would need to develop variety of mutations at the same time to safeguard themselves (Lapresta-Fernández *et al.*, 2012).

Conclusion

Silver nanoparticles within the mean of (27.67± 0.577) are synthesized by the supernatant of *Lactobacillus* once nitrate is more to it. In addition to overcoming resistance and having a lower value than conventional antibiotics, antiseptic activity has shown that AgNPs destroy bacteria at such low concentrations that they do not exhibit acute ototoxic effects on human cells.

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