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Biogenic Silver Nanoparticles Produced by Bacteria Isolated from University Campus Environment Soil Samples in Ras Al Khaimah

[1]Pooja Shivappa, [2]Grisilda Vidya Bernhardt, [2]Sheela Haridas and [3]Michael V Magaogao

[1]Department of Basic Sciences, RAK Medical and Health Sciences University, United Arab Emirates
[2]Department of Biochemistry, RAK Medical and Health Sciences University, United Arab Emirates
[3]Department of Microbiology, RAK Medical and Health Sciences University, United Arab Emirates

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Corresponding Author:
Pooja Shivappa
Department of Basic Sciences, RAK Medical and Health Sciences University, United Arab Emirates
Email: pooja@rakmhsu.ac.ae

Abstract: Silver is observed to be comprised of a huge percentage of silver oxide and the utilization of silver and silver salts is in practice since ancient human civilization. Silver nanoparticles remain to be potential antifungal, antibacterial, anti-inflammatory, and antiviral agents. Further, it is demonstrated that silver nanoparticles have been involved in arresting the growth of several bacterial species thereby reducing their harmful effects. Micro-organisms are being utilized as eco-friendly nano-factories for the synthesis and bio-production of various nano-meter-sized compounds. Metals and micro-organisms are collaborative. Concurrently, micro-organisms are also capable of extracting or accumulating metals. The study suggested an eco-friendly approach for the extracellular synthesis of AgNP using soil-derived actinomyces followed by tracing its efficacy against bacteria. The characteristic of AgNP is the UV-Visible with also the FTIR method. Synthesized nanoparticles are screened for *Streptomyces* antibacterial activity by cross streak method followed by PCR analysis. The particle size distribution per intensity is estimated by the Dynamic Light scattering method. The study also predicted that the hydrodynamic diameter of the particles increases with the increase in the repetition rate. The microbial synthesized AgNP has been observed to possess high toxicity to bacteria with a greater antimicrobial property.

Keywords: Silver Nanoparticles, Silver Nitrate, Actinomyces, FTIR, *Streptomyces*

Introduction

The rapid emergence of nanotechnology accelerated the origin of distinctive and novel nanosized substances. The antimicrobial characteristic of silver are explored by the existing research and found that Silver (Ag) nanoparticles possess great applications in different fields like intercalating materials for optical receptors, electrical batteries, polarizing filters, catalysts in bio-labeling, chemical reactions, bioactive materials and sensors, signal enhancers in immunoassay and antimicrobial agents. These features are due to the microbial colonization correlated with the biomaterial-related infection. The broad spectrum of silver nanoparticles is synthesized by various methodologies and therefore a need arises to frame ecologically friendly methods with non-toxic chemicals. The diversified microbial flora on the earth attracted several researchers for exploring the production of novel Metallo nanoparticles(Khaleghi *et al*., 2019).

The biological production of these nanomaterials has attained more attention due to usage in mild experimental conditions concerning pressure, pH, and temperature (Matei *et al*., 2020). This process could also provide additional advantages over the chemical techniques like minimum cost and maximum productivity. For instance, bacteria on exposure to metals or any other toxic materials beyond a specific level might progress through numerous mechanisms including efflux, changes in toxicity and solubility, extra-cellular complication or metal precipitation, and deficiency of certain metal-transport

systems (Khatami *et al*., 2017; Hamedi *et al*., 2017). In addition, considering the applications of silver nano-particles in several commercialization areas, this study recommends an eco-friendly process to synthesize silver nano-particles through actinomycete isolation from the mangrove soil. In this case, the nano-particles have been extra-cellularly synthesized. This is characterized by FTIR, TEM, and UV-Vis analysis. After synthesis, it has been tested to analyze activity that is anti-bacterial on bacterial strains that are both gram-negative and gram-positive that lead to diseases in humans (Kthiri *et al*., 2021).

One of the most promising technologies in today's scientific systems is Nanotechnology because of its efficient nanoparticles and its wide use in biotechnology, medicine, and chemistry (Prabhu and Poulose, 2012; Sorbiun *et al*., 2018). There is an increasing need for current synthesis protocols to imbibe environmentally conducive nanoparticles that don't contribute to waste that is toxic. (Vahabi *et al*., 2011). Several methods have been developed for nanoparticle synthesis, and the most significant aspect of this technology is related to the production of nanoparticles with clearly defined size, shape as well as a regulated monodispersity (Pugazhenthiran *et al*., 2009). Routes that employ biotechnology have come forth as safer alternative processes for nanoparticle production using the surrounding biomass (Baker *et al*., 2013). There are documented studies that show that such biological methods are economical and environmentally conducive, for synthesizing particles at the nanoscale. *E. coli* (Kannan *et al*., 2010), *Bacillus* cereus (Sunkar and Nachiyar, 2012), Corynebacterium sp. (Arun *et al*., 2013), and Yeast species include the MKY3 yeast strain (Kowshik *et al*., 2002), Saccharomyces cerevisiae BU-MBT CY-1 (Selvakumar *et al*., 2011), fungi included, Aspergillus terreus (Li *et al*., 2011), Aspergillus clavatus (Verma *et al*., 2010) Trichoderma reesei (Vahabi *et al*., 2011), algae Cyanobacteria (Gowramma *et al*., 2015), T. asperellum (Mukherjee *et al*., 2008) and lichen Parmotrema praesorediosum (Mie *et al*., 2014) can absorb and deposit metals and can be used to reduce environmental pollution as well as can recover metal that is in waste. There are also adaptations to environments that are rich in heavy metals that produce microorganisms that exhibit characteristics such as bioabsorption, biopreservation, extra-cellular sequestration, mechanisms of transport, and chelation, and these mechanisms characterized by the resistance are the main proponent for the use of microorganisms in nanoparticle synthesis (Baker *et al*., 2013).

Considering precious metals, silver (Ag) is the preferred metal in such systems of biological nature, including medicine and living organisms (Elavazhagan and Arunachalam, 2011). Numerous studies have shown that nanoparticles can be produced in microorganisms by reduction mechanisms that are either enzymatic or non-enzymatic. Ahmad *et al*. (2003) showed that enzymes responsible for nanoparticle biosynthesis are NADH-dependent. There are also reposts by some researchers that without the intervention of biological enzymes, nanoparticles were generated (Ahmad *et al*., 2003). Liu *et al*. (2000) produced Au^{3+} nanoparticles in dried *Bacillus* megaterium cells (Liu *et al*., 2000). Studies have been reported on the Ag^{+} absorption by some microorganisms (Sneha *et al*., 2010). No enzyme involvement was observed in these cases. This mechanism involving enzymatic reduction indicated that a certain microbial cell wall of organic functional groups may be the cause for the synthesis process under a restrained environment (Lin *et al*., 2001). The biomass that microorganisms produced that dried, such as Lacto*bacillus* A09 and *Bacillus* megaterium D01, also plays a significant role in the reduction of Ag^{+} ions through interactions between Ag^{+} and certain classes in the cell wall of the microbes. (FU *et al*., 2000).

Silver nanoparticles, when compared to all other materials of the nano nature, have proven in their track record to be the antimicrobial agents with the most positive effect and have shown immense possibilities in biomedical applications, especially because of a critical ratio calculated by their surface area-to-volume, which is found to be very large (Bhattacharya and Mukherjee, 2008; Hirst *et al*., 2009), as well as various activities that are biomedical (Hussain and Ferguson, 2006). In particular, with the new developments of research on metal nanoparticles, Ag-NPs are receiving new-found attention as potential antibacterial agents (Baker *et al*., 2005; Firdhouse and Lalitha, 2013).

The present study focused on the synthesis and characterization of silver nanoparticles from actinomycete colonies that are naturally isolated from soil samples in a compound environment by non-enzymatic methods, additionally evaluating the antimicrobial activity of silver nanoparticles against pathogenic bacteria.

Materials and Methods

Yeast extract, potato dextrose, bacto tryptone, silver nitrate ($AgNO_3$), agar agar, sodium hydroxide (NaOH), (ammonium hydroxide) $NH_3.H_2O$ (25% w/w, AR), (Nitric acid) HNO_3, (sodium chloride) NaCl.

Actinomyces Isolation

Soil Samples collected from RAKMHSU were treated with $CaCo_3$ and dried in an oven at 45°C for 1 h. One gram of soil was added into 10 mL of sterile water, mixed, and then serially diluted up to 10^{-3}. From these dilutions, 0.1 mL aliquots were spread into Inorganic Salt Agar (ISP4) and incubated at 28°C for 6-7 days. Typical actinomycetes colonies were sub-cultured on another ISP4 plate and were identified according to standard microbiological techniques. The starch casein nitrate agar plates were prepared and collected cultures were streaked to obtain pure cultures, then incubated at 28°C for 6-7 days.

Preparation of Biomass

The actinomycetes culture was grown in a 250 mL Erlenmeyer flask containing Tryptone yeast extract broth (ISP1). It was kept on a shaker at 35°C at 200 rpm for 4 days. The flask was removed from the shaker and stored at 5 to 10°C to settle the mycelial biomass. The mycelial mass was separated from the culture broth using man filter paper under sterile conditions. Both filtrate and mass were collected. The filtered mass was washed thrice with distilled water. Mycelial mass was then centrifuged at 6000 rpm for 5 min to remove any excess water if any. The obtained mycelial pellets were used for the synthesis of silver nanoparticles.

Synthesis of Nanoparticles

The filtrate and mass were re-suspended in an aqueous AgNO3 solution (0.01 M) and put in a shaker at 37°C for 96 h. The color change was observed from clear to cloudy white to brown. UV Readings were taken in UV-1800, Shimadzu UV spectrophotometer. The filtrate peak was observed at 393 nm while the mass peak was observed at 245 nm. Particle size analysis of mass and filtrate was checked in Anton Paar Litesizer-500 (Particle size analyzer) at a dilution of 1:2. FTIR analysis was performed using FTIR Spectrometer Agilent Cary 630 FTIR.

FTIR Analysis

Using FTIR Spectrometer Agilent Cary 630 FTIR, FTIR analysis was conducted to plot the possible molecules that are associated with nanoparticle formation.

The characterization involved identifying the biomolecules responsible for the bioreduction of Ag^+ ions, and the sample was prepared by using synthesized silver nanoparticles in a dried powder format. The measuring spectrum was by scanning the spectrum in the range of 450–4000 cm^{-1} at the resolution of 4 cm^{-1}.

Screening for Antimicrobial Activity of Streptomyces Isolates

The antimicrobial activity of *Streptomyces* isolates was evaluated by the Cross Streak method. Each of the isolates was streaked on Starch Protein-M Agar as a straight line and incubated at 30°C for seven days. Then the plates were seeded with test organisms (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Bacillus spp*, *Candida albicans*) by a single streak at a 90° angle to the Streptomyces isolates and incubated at 37°C for 24 h to determine whether the test organisms made any further growth towards the master streak.

PCR

The genomic DNA of each isolate was extracted using Hi-PurA Bacterial Genomic DNA Purification Kit (HiGenoMB, India) according to the manufacturer's instructions. A Polymerase Chain Reaction (PCR) was carried out using universal primers (16S rRNA) (5'-GATTAGATACCCTGGTAGTCCAC-3' and 5'-CCCGGGAACGTATTCACCG-3'). Its expected size of PCR product was 602 bp. PCR was performed using Applied Biosystems 2720 Thermal Cycler. The amplification of PCR was used in an initial denaturation at 95°C for a min, 35 cycles of denaturing at 95°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 1 min, and a single final extension at 72°C for 5 min. The purity of the product was determined by electrophoresis in a 1.5% agarose gel. DNA was stained with ethidium bromide and the gels were captured in an imaging system (Bio-Rad Gel Doc EZ Imaging System, USA).

Results

Two bacterial isolates of *Streptomyces Spp* have been cultured in media shown in Fig. 1. The bacterial isolates with antibacterial silver nanoparticles were synthesized successfully followed by tests to determine the antimicrobial activity of the isolates. The change in color from colorless to brown or deep yellow characterized the nanoparticles in the medium and confirmed them.

The molecular characterization and identification of the two isolates of *Streptomyces Spp.* were performed using PCR. The primers for amplification of 16s rRNA of *Streptomyces spp.* isolates 1 and 2 were designed to give a product of size 602 bp. The 16s rRNA genes of EC (*E. coli*), SA (*Staph. aureus*), and BA (*Bacillus spp.*) were used as Positive controls while the CA (*Candida albicans*) and SC (*Saccharomyces cerevisiae)* both yeasts were used as Negative controls. It can be observed from Fig. 2 that *Streptomyces Spp.* isolate 1 and 2 were found to have an amplified band of 602 bp.

The antimicrobial activity of the isolated *Streptomyces spp.* was determined by inoculating the isolates on Starch Protein-M Agar in an as straight line and incubating at 30°C for seven days. Then the plates were seeded with test organisms (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Bacillus spp*, *Candida albicans*) by a single streak at a 90° angle to the *Streptomyces* isolates and incubated at 37°C for 24 h to determine whether the test organisms made any further growth towards the master streak. It was observed from the study that there was partial inhibition of *Bacillus spp.* observed for isolate 1 of *Streptomyces spp.* while there was no antimicrobial effect of the *Streptomyces spp* on the growth of *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *and Candida albicans.* However, isolate 2 of *Streptomyces spp.* showed good antimicrobial activity by showing inhibition in the growth of *Staphylococcus aureus* and *Bacillus spp.* was observed. Figure 3 similar to isolate 1, no inhibition in the growth of *Escherichia coli* ATCC 25922, and *Candida albicans* was observed for isolate 2 of *Streptomyces spp.*

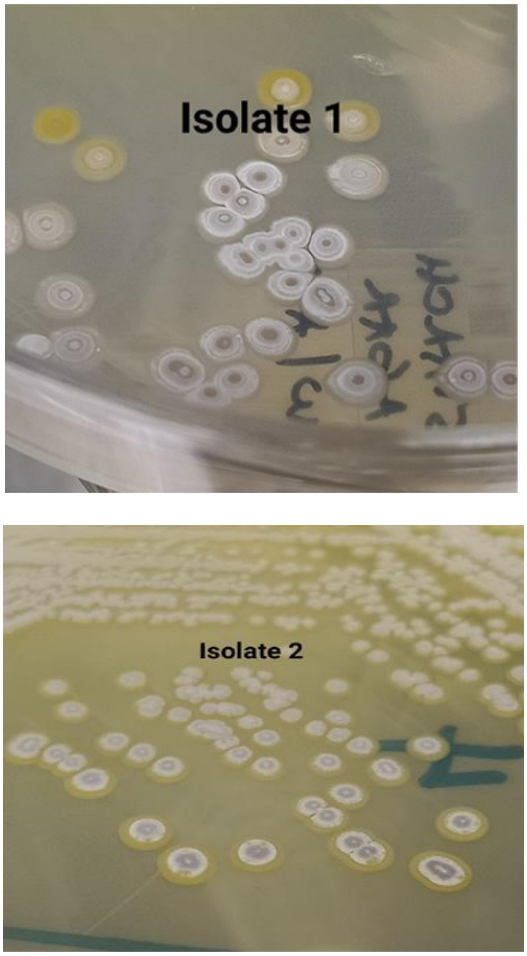

Fig. 1: Figure showing two bacterial isolates of *Streptomyces Spp*

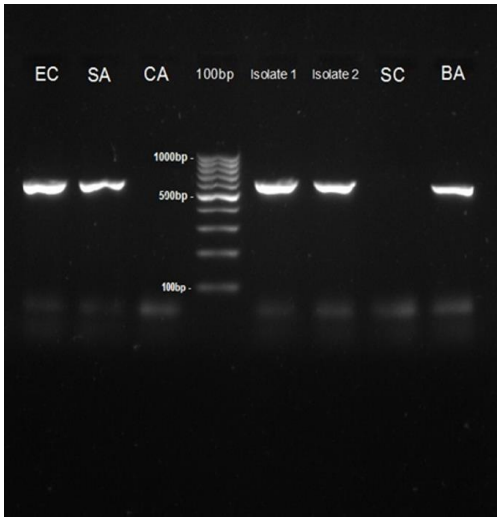

Fig. 2: *Streptomyces* Isolate 1 and 2 detected. EC (*E. coli*), SA (*Staph. aureus*), BA (*Bacillus spp.*) used as Positive controls. CA (*Candida albicans*) and SC (*Saccharomyces cerevisiae)* both yeasts were used as Negative controls. Molecular weight marker 100bp DNA

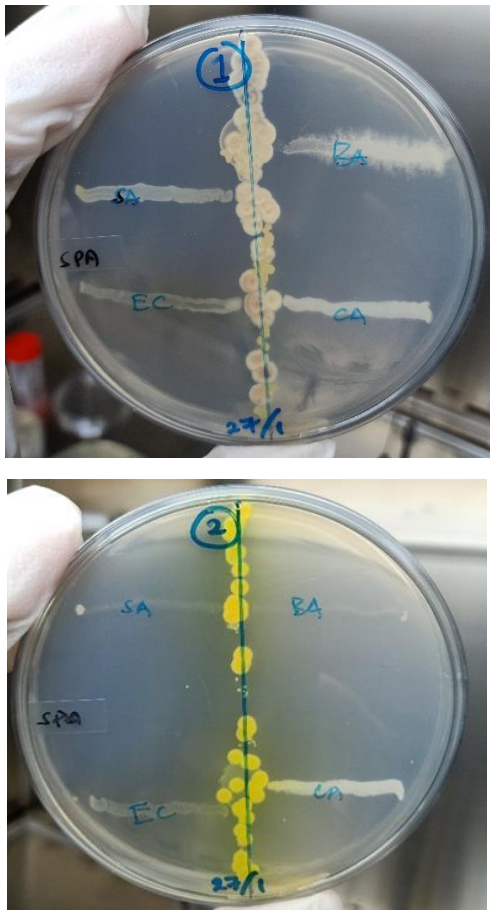

Fig. 3: Isolate 1 showing partial inhibition of *Bacillus spp*. Isolate 2 shows good inhibition of *Staphylococcus aureus* and *Bacillus spp*

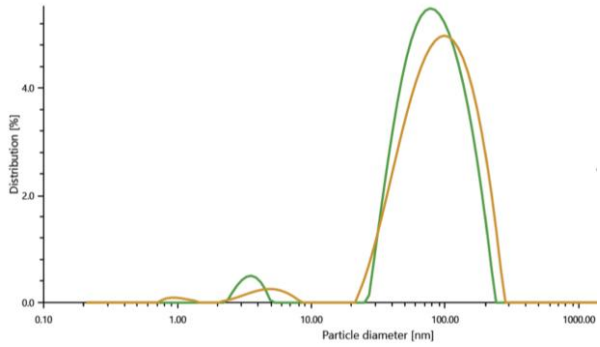

Fig. 4: Figure showing particle size density by intensity using DLS

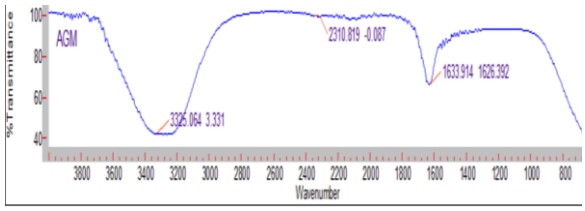

Fig. 5: FTIR spectra of the nanoparticle suspension

Particle Size Distribution and FTIR Characterization

DLS (Zetasizer 4000, Malvern Instruments Ltd, Malvern, UK) was used to measure the size of the nanoparticles using the hydrodynamic diameter and size distribution (polydispersity index, PDI¼m^2/_2). All DLS measurements were carried out at 25ºC with an angle detection of 908. Continually diluted with water, the particle dispersion showed the desired number of counts, where the dilution was done until this was obtained. The number of counts that is high enough to get the highest possible signal-to-noise ratio, yet small enough to prevent multiple scattering to occur is the desired number of counts. Aliquots from each preparation batch were sampled in DLS cuvettes and nanoparticles were then examined for equivalent diameters, size distribution, and Fig. 4. The Ag nanoparticles were detected at 91.2 nm

FTIR analysis was clear from the FTIR spectrum of AgNPs which gave peaks at 3325 cm^{-1} corresponding to the OH stretch of carboxylic acid and 1633 cm^{-1} corresponding to N-H bending of primary Fig. 5. Also reported earlier is that the biological molecules perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium.

Discussion

Although the possible mechanism of nanoparticle formation is still unclear, it has previously been suggested that the formation may be related to proteins in the medium (Gole *et al*., 2001).

Isolated actinomyces bacteria were identified as *Streptomyces* spp., using 16S rRNA analysis. This bacterium showed the ability to reduce Ag^+ to Ag_2O, and the nanoparticles ranged in size from 11 to 16 nm and were found to be slightly aggregated.

Due to the interfering effect of salt, the limitations of the usefulness of silver and silver derivatives are overcome by the use of Ag nanoparticles against microorganisms, which have been proven to be effective antibacterial agents (Estevez *et al*., 2021). Antimicrobial activity was also found in the biosynthetic AgNPs against all the test organisms used in this study. This opens the door to new research avenues where endogenous bacteria can also be used for the green synthesis of nanoparticles. This research will extend to the synthesis of gold nanoparticles, especially since they have become an important part of cancer diagnosis and treatment in the medical field.

The antimicrobial results of this study are in good agreement with previous reports (He *et al*., 2018; Jagtap and Bapat, 2013). The precise mechanism of AgNP antibacterial activity is not yet well elucidated (Roy *et al*., 2019). It is hypothesized that small nanoparticles can easily penetrate the bacterial cell wall and interact with various cytoplasmic biomolecules within the cell. Roy *et al*. (2019) described that the charge and particle size of the biocompounds surrounding the surface play important roles in the binding and penetration of the cell wall between bacterial cells and NPs (Roy *et al*., 2019). At the cell surface, NP attachment to the bacterial cell wall disrupts cell integrity, resulting in cell death. This is achieved by inhibiting membrane permeability and respiration function through nanoparticle-induced membrane depolarization (Abalkhil *et al*., 2017; Krishnaraj *et al*., 2010). Previous reports have shown that Ag NPs can penetrate the cell wall following bacterial cell wall damage. AgNPs can affect the function of important biomolecules such as DNA, proteins, lipids, and respiratory enzymes that induce oxidative stress and release ROS and nucleic acid and protein damage by triggering bacterial cell death (Kaushal *et al*., 2015). Similar antibacterial properties of Ag NPs were also described. In their study, they showed that nanoparticles can penetrate bacterial membranes and inhibit specific cell growth, which ultimately leads to cell death. (Li *et al*., 2010). Similar studies by Castro-Mayorga *et al*. (2018) isolated saline soil bacteria, and screened for the silver NP synthesis the cultures from the Baramathi region were investigated in this study and the developed AgNP depicted peaks at 420 to 430 nm for further characterization. The chosen culture has been detected with the utilization of genotypic and phenotypic characteristics and is observed to be B. cereus. The NP characterization has been performed using UV-visible, XRD, FTIR, and SEM. The synthesis depicted characteristic absorption peaks at 433 nm. Further, the FTIR revealed the existence of amino-methyl carbonyl and stretching groups. The corresponding antibacterial activity has been founded to act against human pathogens.

In the inference of (Juan *et al*., 2017) this study made clear that AgNPs effects overall community structure, bacterial abundance, and nitrifying bacteria. There was dosage-dependent relation among NOB and AOB and AgNPs populations. The bacterial phyla's responses are interconnected with their dosage to AgNPs. The result of FCM analysis is Ag^+ on staind N. And AgNps and Europaea are combined with TEM and SEM analysis of exposed. Europa-ea describes the AgNps toxicity on N.europaeasis by Ag^+ dissolution and this is not the sole mechanism. Another study (Abdulsada *et al*., 2021) investigates the effects of stabilization of lime on the transformation and fate of AgNPs. It also evaluates transformation in diversity and population of related bacterial phyla data after implementing stabilized lime sludge which includes AgNPs. This study was conducted by spiking an environment-related concentration of silver nanoparticles in sludge, when stabilized lime was applied

it increases pH by more than 12 within two hours, and also treated lime was applied to soil samples. TEM and EDS were sued for investigating compositional and morphological changes of AgNPs during the process of lime stabilization.

After stabilized lime application sludge into the soil, these soil samples were analyzed periodically for entire genomic DNA and changed in BPD ("bacterial phyla diversity") with qPCR ("Quantitative polymerase chain reaction"). The final results show that lime is treated effectively and eliminated AgNPs from the phase of aqueous and silver nanoparticles were stored on molecules of lime. These results reveal that AgNP does not impact the diversity and presence of phyla in the soil. Moreover, stabilized lime sludge towards silver nanoparticles impacted the abundance of every phylum over the period. No important effects on TOC, percentage of living cells, and HPC were observed.

Sorbiun *et al*. (2018) focussed on the AgNP synthesis from brevis followed by the characterization of several spectroscopic and microscopic methods that confirmed the SPR peak at 420 nm and the range of 41 to 68 nm with a spherical shape. The existence of bioactive compounds in silver nanoparticles against MDR pathogens like *Salmonella typhi and Staphylococcus aureus* and these bioactive materials can be used as better antimicrobial agents for efficient disease management.

Conclusion

Nanotechnology possesses a vital role in the advancement in the area of scientific research and technology. It deals with the manipulation, production, and utilization of nano-sized materials. These substances provide the most precise addressing numerous unresolved issues of mankind. Prior understanding regarding the assembly of small structures could lead to the infinite possibilities of improved structures, devices, and materials thereby bridging the gap between the synthesis of nanoparticles from microbes and its practical implementation in different fields. Hence extensive interdisciplinary knowledge has to be explored and integrated for the beneficial activity of living beings. In accordance, nanomaterials are utilized as active catalysts efficiently for the oxidation and deoxidation of organic substances.

Existing literature represented nanoparticles as active substances for photocatalytic degradation of toxic chemicals and organic dyes. Apart from that recent research in the inorganic nanomaterials with enhanced anticancer and antimicrobial properties created a new gate in the medical and pharmaceutical industries. Accordingly, metal oxide nanoparticles have been proved to be an extraordinary agent in the framing of nanoscale setup for therapeutic applications. The information from existing literature will not provide adequate details and hence for such exploration and huge scientific scope in the analysis of biological applications of silver nanoparticles the presented study has successfully synthesized silver nanoparticles and carbon doped zinc oxide nanoparticles by various methods. The developed nanoparticles are characterized by FTIR and UV-Vis techniques. In the present study, silver nanoparticles are synthesized from mycelial pellets of actinomyces and precipitated from a silver nitrate solution. The inhibition concentration showed a better efficacy for *Streptomyces* species. The present findings not only confirm bacteria isolated actinomyces with silver nanoparticles but also indicate the effective antibacterial properties of the silver nanoparticles which possess greater therapeutic applications.

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Author's Contributions

Pooja Shivappa and Grisilda Vidya Bernhardt: Conception, design, and drafting, finalized the article.

Sheela Haridas and Michael V Magaogao: Collection of sample and methodology carried out in the analysis.

Ethics

The institutional ethical committee RAKMHSU-REC-161-2021/22-F-M, approved the study.

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