Original Article

Antimicrobial Activity of Silver Nanoparticles Biosynthesized by the Fungus *Byssochlamys spectabilis* Against *Actinomyces dentalis*

Ali Rahman Al-Shujairi, Azher Abdulhafidh Jabir[1], Nebras N. Al-Dabagh[1]

Department of Biology, College of Sciences, Babylon University, Al-Mahawil City, Babylon Province, [1]Department of Microbiology, College of Dentistry, Babylon University, Babylon Province, Iraq

Abstract

Background: Over time, pathogenic microorganisms have become more resistant to antimicrobials, and this is a risk to human health. Therefore, alternatives that are more effective against these pathogens must be searched for. **Objectives:** This study, which was completed in the College of Dentistry and College of Science at the University of Babylon, aimed to produce silver nanoparticles in a biological method using a fungal extract of this *Byssochlamys spectabilis* fungus, for the first study in Iraq. **Materials and Methods:** Where it was studied properties its morphology, microscopic, and molecular diagnosis by PCR and documented in the International Information Bank (NCBI) under the isolate name "Al-Rahman" and has Accession number (MN318956.1), as well as studying the fit environmental conditions, where it gave The best growth is at 27°C temperature, pH = 6.5, and the best type of culture medium is PDA. This fungus was used as a reducing agent, very fitting for the synthesis of AgNPs and its use as an antimicrobial. **Results:** The color change of AgNPs from yellow to ruddy brown was the principal Sign of biosynthesis. UV-noticeable spectrophotometry was utilized to portray biosynthetic nanoparticles and the greatest ingestion pinnacle of the nanoparticles (420 nm) was noticed. The size and distribution of nanoparticles were determined by using scanning electron microscopy (atomic force microscopy [AFM] and transmission electron microscopy [TEM]), and the shape was circular and homogeneous, the size varied from (10.49-16.42 nm) to test these fabricated nanomaterials. The isolated *Actinomyces dentalis* from patients who had periodontitis and who visited specialized dental centers and private clinics at the University of Babylon in the College of Dentistry, after culturing this sample in the laboratory. Different concentrations of the nanomaterial (31.25, 62.5, 125, 250, and 500 µg) were prepared and its effect as an inhibitory agent against this isolate was studied. It was 125 µg the best concentrate to kill *A. dentalis*. **Conclusion:** Silver nanoparticles biologically synthesized by *B. spectabilis* were able to inhibit bacterial growth of strain of medical importance, which was demonstrated by obtaining inhibition percentages greater than 20 cm of *A. dentalis*.

Keywords: *Actinomyces dentalis*, antimicrobial activity, biosynthesis, *Byssochlamys spectabilis*, fungal extract, periodontal abscess, silver nanoparticles

Introduction

A good researcher should search for alternatives that are low cost, more efficient, stable with the time, fit with biological systems, and eco-friendly, especially about the emergence of new strains of microorganism that causes diseases that are resistant to antimicrobial. Silver has medical properties of been known since the nineteenth century, and silver has been used in many antimicrobial applications. This detail of silver makes it an ideal choice for some jobs in the clinical field. Silver is regularly utilized in nitrate structure to prompt antimicrobial impacts, the

Access this article online

Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_1531_23

Address for correspondence: Mr. Ali Rahman Al-Shujairi, Department of Biology, College of Sciences, Babylon University, Al-Mahawil City, Babylon Province, Iraq.
E-mail: sci.ali.shakir.lec@uobabylon.edu.iq

Submission: 08-Oct-2023 **Accepted:** 16-Dec-2023 **Published:** 30-Apr-2026

How to cite this article: Al-Shujairi AR, Jabir AA, Al-Dabagh NN. Antimicrobial activity of silver nanoparticles biosynthesized by the fungus *Byssochlamys spectabilis* against *Actinomyces dentalis*. Med J Babylon 2026;23:614-20.

silver nanoparticles are utilized to a monster increment surface region accessible for microbials to be presented to. Notwithstanding, silver nanoparticles are utilized in numerous antibacterial applications.[1]

Drug resistance of microbial is a severe problem to public health and constitutes a challenge to researchers worldwide for search new and good antimicrobial.[2]

Nanoparticles (NPs) have an active function in the expansion of new antibacterial materials to eliminate a numeral of pathogenic bacteria.[3]

Nanotechnology has been used in the field of prosthodontics to enhance the property of a material used. Nanotechnology includes a range of advances, procedures, and cycles that can manage a matter at the nanoscale in the middle of between 1 and 100 nm in size.[4]

The current advancements in nanotechnology use silver nanoparticles widely as a novel medicinal agent as antimicrobial and anti-cancerous agents.[5]

Compared to classic antibiotics, AgNPS have antimicrobial effects without the generation of resistance to microbial.[6]

Silver nanoparticles AgNPs altogether improve bactericidal characterize when gotten together with antibacterial agents, including ceftazidime, cefotaxime, meropenem and ciprofloxacin. Inactive serum harms major areas of strength for reestablishing improvement against multi-safe bacterial strains when they have coexisted with AgNPs.[7]

The effects of antimicrobial depend on multipole factors as a size (the small of nanoparticles have very toxicity), surface, surface charge, shape, and solubility.[8]

Because of the expansive use of AgNPs in different fields, as referenced above, the interest in huge scope, eco-accommodating, non-dangerous, quicker, and biocompatible creation of AgNPs with explicit size, shape, and properties has likewise expanded. AgNPs can be arranged either by physical, synthetic, or natural techniques.[9]

Biosynthesis utilizes harmless to the ecosystem green science processes using organic substances, for example, microorganisms, that will go about as natural plants. It offers perfect, biocompatible, non-harmful, and harmless to the ecosystem techniques, which can create a large number of shapes, sizes, structures, and physicochemical properties.[10]

However, a clever natural strategy for the intracellular and extracellular blend of silver nanoparticles utilizing organisms. Parasites are a suitable option in AgNP biosynthesis because of their capacity to deliver bigger measures of nanoparticles contrasted with microorganisms. This is a result of their capacity to deliver bigger measures of proteins that are straightforwardly corresponded with the improvement of AgNP creation.[10]

Fungi has superb potential for the development of many mixtures that can be utilized in various applications. Around 6400 bioactive substances are known to be created by imperfect filamentous growths (ascomycetes and blemished organisms) and other fungi species.[11]

Byssochlamys spectabilis Endophytic fungus (asexual state Paecilomyces variotii) has been classified in the Trichocomaceae family.[12]

Byssochlamys species produce ascospores that are heat-safe and endure extensive periods in heat over 85°C. Notwithstanding their intensity opposition, Byssochlamys species can develop under exceptionally low oxygen pressures and can shape pectinolytic enzymes.[13]

The combination of physiological characteristics makes the Byssochlamys species very important in many antimicrobial applications.[14]

Periodontitis is the most widely recognized oral disease. A persistent disease is described by the obliteration of tissues. It is brought about by multifactorial etiology.[15]

Silver nanoparticles combined with a proper covering expert can propel the limitation influence against gram-negative microorganisms that predominantly cause periodontal pollution. All the more unassumingly assessed AgNPs presented the higher antimicrobial property against oral anaerobic pathogenic minute living beings. Since a coordinated tissue recuperation layer with silver nanoparticles diminished adherence and entry of infinitesimal organic entities, treatment of intra-hard flaws using coordinated tissue recuperation film with silver nanoparticles can chip away at clinical accomplishment.[16]

Periodontal dressing covered with silver nanoparticles can be utilized to treat gingival injuries. This treatment speeds up the beginning stages of twisted recuperating, with evidently new collagen amalgamation and neovascularization. In any case, some silver nanoparticles can incite irritation and unfamiliar body responses in periodontal dressing.[17]

This review, we report a biosynthesis of AgNPs by the fungus of *B. spectabilis*. The attributes of integrated AgNPs were distinguished by UV-apparent spectrophotometer, Fourier transform infrared spectroscopy (FTIR), X-beam diffractometer (XRD), atomic force microscopy (AFM), and transmission electron microscopy (TEM). The antimicrobial action of AgNPs was likewise tried. This is the primary report in Iraq that utilized *B. spectabilis* as a lessening specialist in the biosynthesis of AgNPs.

Materials and Methods

Fungal strain

A biological synthesis of AgNPs was done using the fungal isolation (*B. spectabilis* strain ALRAHMAN1) which was

previously isolated and identified, that has Accession number (MN318956.1) in GenBank for NCBI site, preserved in the advanced fungi Laboratory, University of Babylon, Faculty of Science, Department of B iology.

Bacterial isolate

The clinical isolate of bacterial species (*Actinomyces dentalis*) was isolated from the patients who have periodontal infections. The sample was gathered from subgingival dental biofilm by embedding three sterile paper focuses by 5 mm profundity into the periodontal pocket for 30 s and handled by Loberto *et al*. From that point onward, all paper point tests were quickly handled in clean saline and sequentially weakened 10 folds in 1 mL thioglycollate stock (Code No. CM0173, Oxoid). Tests were moved to a microbial lab right away, and fifty µl of weakened subgingival examples were spread on the outer layer of the plates containing Mitis Salivarius agar (Feline. No. 01337, Sigma-Aldrich). Then brooded in a candle container at 37°C for 48 h. From that point forward, the recuperated provinces on the specific agar media were exposed to removed genomic DNA by PCR method and recognized microscopically, examination of arrangements with kept groupings of in quality Bank data set (NCBI). In this study samples were obtained from the specialized dental centers in the College of Dentistry at the University of Babylon.

Biomass preparation

Byssochlamys spectabilis isolate were the grown in flasks (250 mL), all flask containing (100 mL) from liquid medium Potato Dextrose broth (PDB), this isolate incubated in Incubator shaker at 28°C and agitated at (150 rpm) for 5 days. After incubation period, fungal biomass harvested through Whatman No. 1, later thoroughly wash with deionized water to remove the effect of culture media from the biomass. After that, 20 gm of soft biomass with deionized water (flask 250 mL) was incubated at 28°C for 3 days and agitated at 150 rpm. Finally, the biomass was filtrated using Whatman No. 1. Fungal filtrate was used for manufacture of nanoparticles.

Extracellular synthesis of AgNPs

In order to manufacture nanoparticles from the extracted fungal filterate, (50 mL) of fungal filterate was a mixed with (50 mL) of $AgNO_3$ solution. The mixture was place in flask (250 mL) incubated at 28°C for 3 days. The reaction mixture without silver nitrate was used as control. All mixtures were kept in the dark to ovoid light events during the experiment. Finally, AgNPs were centrifuged at (10,000 rpm) for 10 min, repeated twice, and collected for further characterization.

Characterization of AgNPs

Spectrophotometric analysis

The first step in the characterization of silver nanoparticles utilizing the U-noticeable spectroscopy is the decrease of silver nitrate in the arrangement and the development of AgNPs was, to begin with, affirmed by visual perception of variety change from pale white to ruddy brown and further affirmed by UV-apparent spectroscopy. A little aliquot (2 mL) of the shaded suspended particles was taken in a quartz cuvette and noticed for frequency filtering running between 190 and 1100 nm at various time stretches with refined water as a kind of perspective utilizing the twofold shaft spectrophotometer (Shimadzu, UV-1800 Å).

(FTIR):Fourier transform infrared spectroscopy

Chemical composition of synthesized AgNPs was concentrated by FTIR (Perkin/Elmer WQS-530 Rayleigh spectrometer) that portrayed in the reach 4000–400 cm^{-1} utilizing KBr pellet technique.

Atomic force microscopy (AFM) and TEM

TEM and AFM portrayal gave further understanding into the morphology and molecule size dissemination profile of the different nanoparticles that were encouraged by centrifugation [Figure 4]. The examination of micrographs utilizing RASTAK LAB programming uncovered that the silver nanoparticles were basically circular in shape with sizes running between 10.49 nm (the littlest nanoparticles) and 17.42 nm (the biggest nanoparticles), average size 11.1 nm.

Assessment of antimicrobial activity (agar well diffusion method)

A suspension of the *A. dentalis* were cultivated on Mueller Hinton Agar for antimicrobial susceptibility testing. A 0.1 mL of bacterial suspension was added to culture media and spreading it by using spreader, and making three replicates from Petri dishes. Thereafter, made five holes in the culture media by using the cork borer. A 1 mL of the previously prepared different concentrations from (AgNPs) were added to the four holes, the plates were incubated at 37°C for 24 h, anaerobic candle jar and then the diameter of the inhibition zone was measured.

Ethical approval

The study was directed as per the moral rules that have their starting point in the Announcement of Helsinki. It was completed with patients verbal and logical endorsement before test was taken. The review convention and the subject data and assent structure were checked on and endorsed by a neighborhood morals council as per the record number 865 for (March 7, 2022) to get this approval.

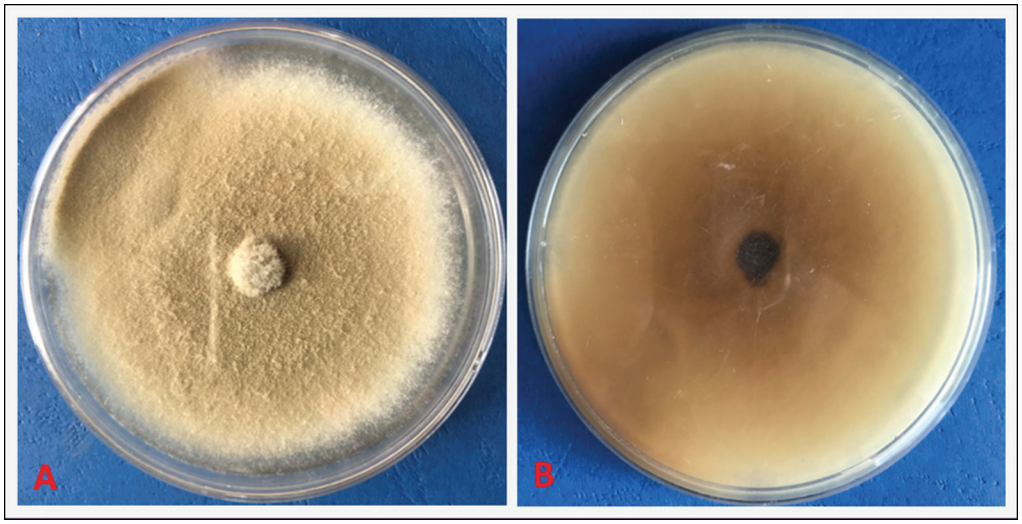

Figure 1: Colony of *Byssochlamys spectabilis* (A: upper side, B: revers side)

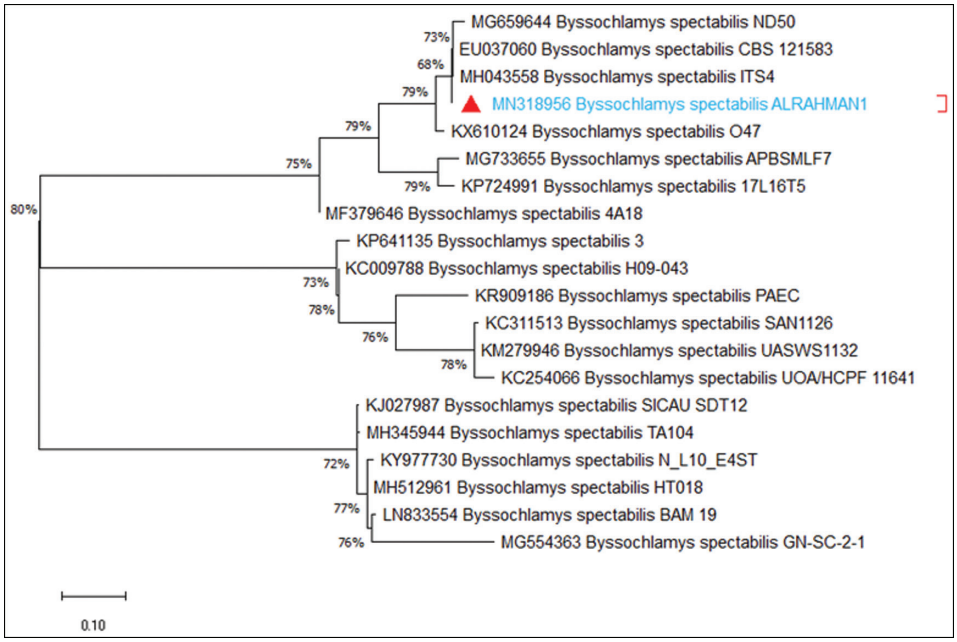

Figure 2: Phylogenetic tree of 20 *Byssochlamys spectabilis* strains based on 18S rRNA gene sequences

Results

Nanotechnology is developing quickly in the field of biological science. In this study, AgNPs were successfully synthesized by *B. spectabilis* cell filtrate. The cultured on PDA medium at 27°C. Based on the characteristics of colony morphology [Figure 1] the strain was identified as a member of *Byssochlamys* genus. In addition to morphological identification, molecular identification was also applied. The 18S ribosomal RNA gene is (100%) homology to *B. spectabilis* (accession number MN318956.1) according to the Gen Bank of database NCBI. A phylogenetic tree was created by MEGA 5.2 with its nucleotide sequence to check homologs from different fungi [Figure 2]. According to morphological and molecular characteristics, the strain was identified as *B. spectabilis*. Many *Byssochlamys* have been reported to be able to synthesize silver nanoparticles, due to their ability to produce abundant secondary metabolites 15. This is the first report in Iraq of the synthesis of AgNPs by *B. spectabilis* using extracellular supernatant in an eco-friendly manner.

AgNPs biosynthesis and characterization:

When $AgNO_3$ was added to the *B. Spectabilis* extract, the color changed from yellow to brown ($AgNO_3$ framed) [Figure 3]. This peculiarity is because of the surface Plasmon reverberation of AgNPs. Different response time (1-3 days) of the combination cycle was identified and observed

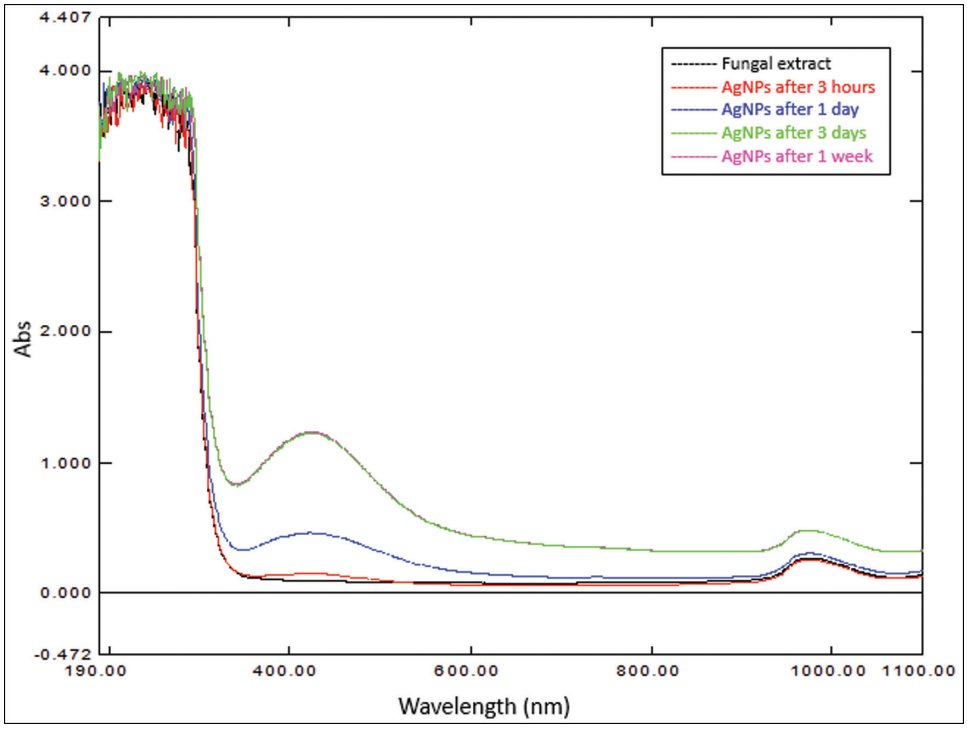

Figure 3: UV–visible absorption spectrum of AgNPs synthesized by *Byssochlamys spectabilis* with different time

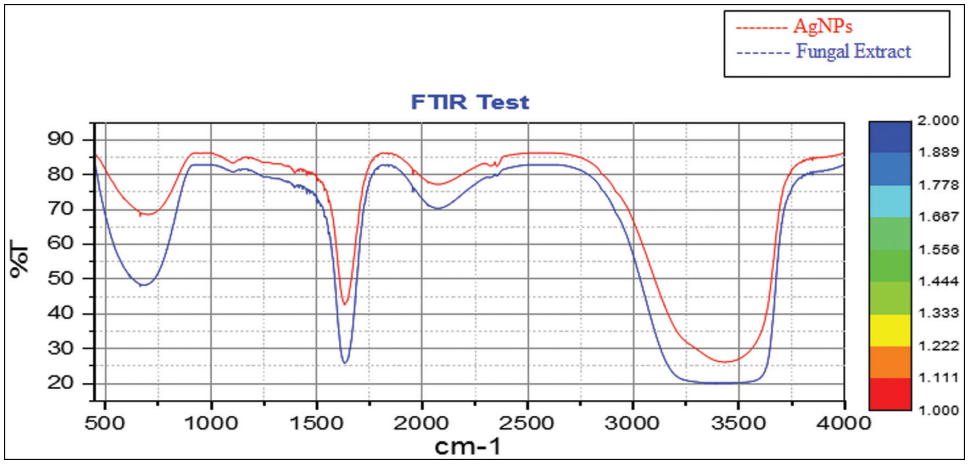

Figure 4: FTIR spectra of the compound fungal extract and biosynthesized AgNPs

by UV-noticeable spectrophotometer with an examining range going from 190 to 1100 nm. As displayed in Figure 4, a solid absorbance top at 420 nm was recognized, which demonstrated the presence of AgNPs. From the UV results, we can see that as the hour of response builds, the most extreme ingestion top at 420 nm additionally increments. This demonstrated how much combined AgNPs is expanding bit by bit in a period subordinate style.

FTIR measurements were done to recognize the major utilitarian gatherings in the Contagious concentrate and their conceivable association in the union and adjustment of silver nanoparticles. The range of Contagious concentrate and combined AgNPs is addressed in Figure 4. Parasitic concentrate showed a few pinnacles demonstrating the mind-boggling nature of the organic material. The groups showing up at 3399.54, 163594, 1505.85, 1455.92, and 11106.08 cm^{-1} were allowed to extend the vibration of O–H obligation of alcohol or phenols, C alkenes, nitro compounds, C=C fragrant, ketones, individually. There was a change in the peaks in combined silver nanoparticles which recommends that practical gatherings of contagious concentrate partake in the development of AgNPs.

By the TEM of an arrangement affirmed the blend of AgNPs and showed that they have a circular shape [Figure 5]. The micrographs obtained by AFM were dissected in the SPIP program verifying that the normal measurement for AgNPs related to 11.1 nm [Figure 6].

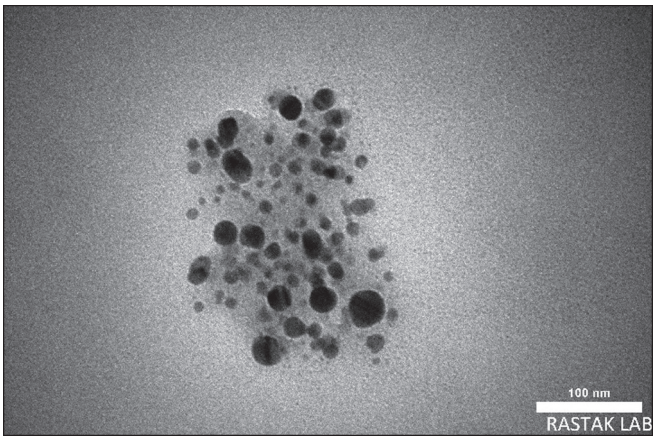

Figure 5: TEM micrograph shows spherical shaped of AgNPs

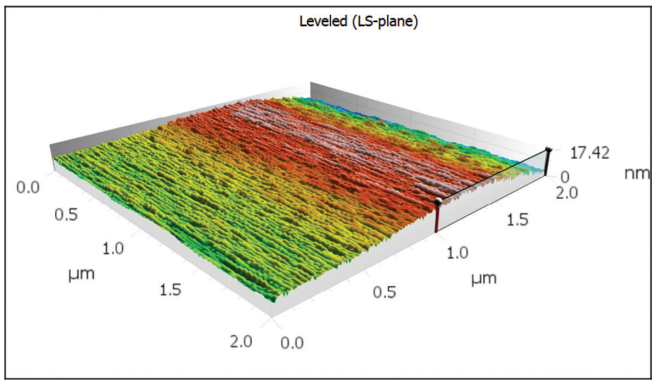

Figure 6: The atomic force microscopy of silver nanoparticles formed by the reduction of silver ions using *Byssochlamys spectabilis* extract

Effect different concentrations of AgNPs on *Actinomyces dentalis* growth

The results of the current review showed that there were tremendous contrasts between the convergence of silver nanoparticles at 500 µg. Focus was the most thought hindrance to the pace of 24 mm, while the fixation was 62.5, 125, and 250 µg. The restraint zone was 10, 16, and 20 mm; the focus 31.25 µg non-inhibitory focuses, as displayed in Figure 7.

Discussion

Silver has been involved starting from the primary developments as a broadspectrum antimicrobial, fundamentally for water sanitization and consumes, in light of the fact that its harmful impact is a less for the human cells than for microorganisms. These days its still broadly utilized silver sulfadiazine a most utilized compound, but it have as of now settled on its utilization in nanoparticles in light of the fact that antimicrobial impact of silver changed over into NPs is 10.49–16.42 more grounded than the impact of silver particles alone since a 100 nm nanoparticle can contain around 10,000 to 15,000 silver molecules; and the more modest the molecule size the impact is more noteworthy, in light of the fact that

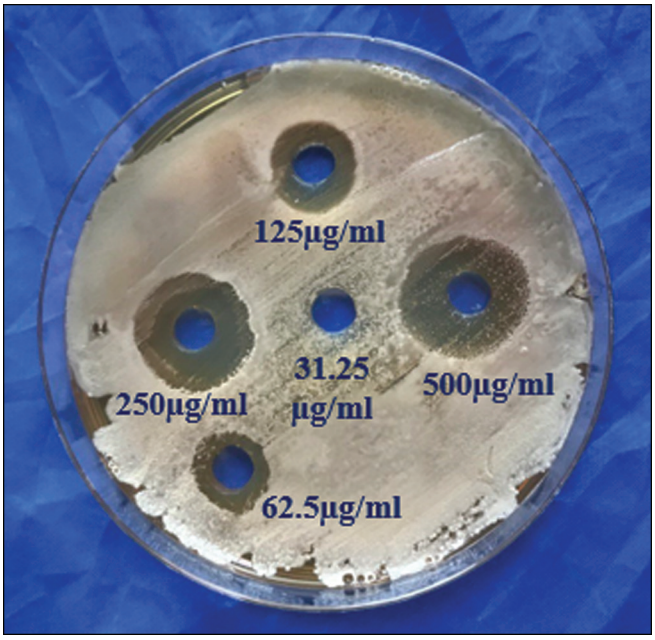

Figure 7: Antibacterial activity of AgNPs at different concentrations

they have bigger contact surface, which communicates with the microbial cell film, modifying a few capabilities like porousness and breath. The AgNPs utilized in this study had a typical measurement of 10.49 nm, so the little size got could be one of the elements that permitted them to get a more noteworthy antimicrobial impact contrasted with different examinations.[5,8,18,19]

Conclusion

Silver nanoparticles biologically synthesized by *B. spectabilis* were able to inhibit bacterial growth of strain of medical importance, which was demonstrated by obtaining inhibition percentages greater than 20 cm of *A. dentalis*. In every one of the techniques utilized for the assessment, a more noteworthy impact was made at higher groupings of AgNPs. Because of the great rates of hindrance got in this review, and being harmless to the ecosystem philosophy, by not involving poisonous specialists for the decrease and adjustment of nanoparticles, AgNPs blended by *B. spectabilis* could be a powerful option to nanoparticles orchestrated by chimical and physical methods

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Prabhu S, Poulose EK. Silver nanoparticles: Mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. Int Nano Lett 2012;2:1-10.

2. Silva MRO, Sena, KX, Gusmão, NB. Secondary metabolites produced by endophytic fungus Paecilomyces variotii Bainier with antimicrobial activity against Enterococcus faecalis. Curr Res Topics Appl Microbiol Microbial Biotechnol 2009:519-522.
3. Mohammed HA, Sahi NM, Ahmed RT, Al-Rubaye A. Antimicrobial activity of some nanoparticles synthesized by laser ablation technique against some bacteria isolated from oral cavity. Med J Babylon 2022;19:601-8.
4. Khairi AW, Naji GA-H. The effect of silica nano particle derived from natural rice on surface roughness and wettability of high impact heat cure resin. Med J Babylon 2023;20:308-14.
5. Jain N, Bhargava A, Majumdar S, Tarafdar JC, Panwar J. Extracellular biosynthesis and characterization of silver nanoparticles using Aspergillus flavus NJP08: A mechanism perspective. Nanoscale 2011;3:635-41.
6. Siddiqui A, Deepa D, Mehrotra S, Sharma A. Metabolomics in periodontal disease-A mini review. Dent J 2022;4.1:13-9.
7. Panáček A, Smékalová M, Večeřová R, Bogdanová K, Röderová M, Kolář M, *et al*. Silver nanoparticles strongly enhance and restore bactericidal activity of inactive antibiotics against multiresistant Enterobacteriaceae. Colloids Surf B 2016;142:392-9.
8. Alexandra, COD, Gabriela, RAN. Antimicrobial effect of silver nanoparticles synthesized by Paecilomyces variotii. In: 2019 IEEE International Conference on Applied Science and Advanced Technology (iCASAT), Queretaro, Mexico. IEEE; 2019. p. 1-6. doi: 10.1109/iCASAT48251.2019.9069536.
9. Jalal M, Ansari M, Alzohairy M, Ali S, Khan H, Almatroudi A, *et al*. Biosynthesis of silver nanoparticles from oropharyngeal Candida glabrata isolates and their antimicrobial activity against clinical strains of bacteria and fungi. Nanomaterials 2018;8:586.
10. Warsito, MF. The prospect of microorganism in the silver nanoparticles biosynthesis to enhance antibiotic drug activity as an alternative solution to combat resistances. In: IOP Conference Series: Earth and Environmental Science. IOP Publishing; 2020. p. 012032. Available from: https://iopscience.iop.org/article/10.1088/1755-1315/591/1/012032/pdf.
11. Guilger-Casagrande M, de Lima R. Lima, Renata de Synthesis of silver nanoparticles mediated by fungi: A review. Front Bioeng Biotechnol 2019;7:287.
12. Houbraken J, Varga J, Rico-Munoz E, Johnson S, Samson RA. Sexual reproduction as the cause of heat resistance in the food spoilage fungus Byssochlamys spectabilis (anamorph Paecilomyces variotii). Appl Environ Microbiol 2008;74:1613-9.
13. Samson RA, Houbraken J, Varga J, Frisvad JC. Polyphasic taxonomy of the heat resistant ascomycete genus Byssochlamys and its Paecilomyces anamorphs. Persoonia 2009;22.1:14-27.
14. Rodrigo S, Santamaria O, Halecker S, Lledó S, Stadler M. Antagonism between Byssochlamys spectabilis (anamorph Paecilomyces variotii) and plant pathogens: Involvement of the bioactive compounds produced by the endophyte. Ann Appl Biol 2017;171:464-76.
15. Yin IX, Zhang J, Zhao IS, Mei ML, Li Q, Chu CH. The antibacterial mechanism of silver nanoparticles and its application in dentistry. Int J Nanomedicine 2020;15:2555-62.
16. Chi M, Qi M, A L, Wang P, Weir M, Melo MA, *et al*. Novel bioactive and therapeutic dental polymeric materials to inhibit periodontal pathogens and biofilms. Int J Mol Sci 2019;20:278.
17. Prasetyo BC, Sugiharti RJ, Mahendra I, Halimah I, Widyasar EM, Rusminah N, *et al*. Evaluation of silver nanoparticles addition in periodontal dressing for wound tissue healing by 99mTc-ciprofloxacin. J Young Pharm 2019;11:17.
18. Shrivastava S, Bera T, Roy A, Singh G, Ramachandrarao P, Dash D. Characterization of enhanced antibacterial effects of novel silver nanoparticles. Nanotechnology 2007;18:225103.
19. Das VL, Thomas R, Varghese RT, Soniya EV, Mathew J, Radhakrishnan EK. Extracellular synthesis of silver nanoparticles by the Bacillus strain CS 11 isolated from industrialized area. Biotech 2014;4:121-6.