Extracellular synthesis of silver nanoparticles by *Acinetobacter baumanii* and antibacterial characterization

Nawfal Hussein Aldujaili[1], Fatima Hamzaa Alzubaidy[2], and Ali Abdul Hussein[3]

[1, 2]Department of biology, faculty of science, Kufa University, Iraq
[3]Dep.of Analytical Tech., faculty of Medical and Healthy tech., university of Alkafeel, Iraq.

*Corresponding author: nawfal.aldujaili@uokufa.edu.iq(Aldujaili)

Summary
Bioproduction approaches of nanoparticles get a very important field with eco-friendly and economic welfares over physical & chemical methods of production. The purpose is the biosynthesis of AgNPs from non-pathogenic bacterial isolate. *Acinetobacter baumanii*. Identification to species-level was determined by Polymerase Chain reaction (PCR) with universal primers, the 16S rRNA gene was subjected to nucleotide sequencing. AgNPs were biosynthesized by adding silver nitrate (AgNO3) into the supernatant of *A. baumanii* at 10 mM. AgNO3 was used as a precursor for the synthesis of AgNPs. Biological AgNPs were originally shown by change the color, yellow to reddish-brown. The categorization of AgNPs accomplished by SEM, XRD, and AFM. SEM exhibited well-dispersed AgNPs, homogenous with a diameter of 18-30nm, with inconstant shapes mostly spherical form. XRD detected the size of AgNPs was 21 nm and the AFM showed the three-dimensional structure of AgNPs and the diameter was 99.82 nm. AgNPs displayed antibacterial action to MDR of *E.aerogens, S.aureus, E.coli, S.typhi, K. Pneumonia, P.auroginosa, P.mirabilis, P.Agglomerans*, and *S.pyogenes*.

Keywords: Biogenic silver nanoparticles, *A. baumanii,* Antimicrobial Activity, biosynthesis

How to cite this article: Aldujaili NH, Alzubaidy FH, Abdul Hussein A (2020): Extracellular synthesis of silver nanoparticles by Acinetobacter baumanii, Ann Trop Med & Public Health; 23(S18): SP231804. DOI: http://doi.org/10.36295/ASRO.2020.231804

Introduction

Nanobiotechnology is a novel ground of synthesis and application of nanoparticles with 1-100nm in size [1, 2]. Nanoparticle formulas of metals as Pt, Ag, and Cu & Au had inhibition against pathogens. AgNPs had likely presentations in Nanomedicine and have been permitted for bactericidal actions against infections and avoidance biofilm creation as well as their sole chemical, electronics and optics [3, 4]. Nanoparticles had exclusive features including antibacterial possessions, magnetic, electronic, optical possessions & catalytic actions [1, 5]. Chemical & physical techniques were established for the making of AgNPs. The green creation is an eco-friendly and cheap bio creation technique for AgNPs. The bio creation way of manufacture is proficient with bacteria, fungi or plant sources. Bacterial metabolites certify speedy & dependable reduction of Ag ions to element. The microbial extract has occasioned in production nanomaterials with specific shapes, size & morphology [6, 7]. The extracellular creation of AgNPs using *A. baumannii* species seems to be cheap and eco-friendly [8, 9, 10] to improve real antimicrobial mediators that overwhelmed the MDR of microbes [11]. The study is aimed at synthesing AgNPs with *A. baumannii* and tests antimicrobial activity.

Methods

Culture of *A. baumannii*

The *A. baumannii* was obtained from a higher student laboratory in the faculty of science. BHI was inoculated with *A. baumannii*, incubated at 37°C, 24 hrs, and aerobic condition. *A. baumannii* was identified based on biochemical and morphological tests [12]. The second activation was worked from the first activation and incubated aerobically at 37°C for 24 hrs [9].

Preparation of cell free supernatant of *A. baumannii*

After 24 hrs, the culture was subjected to 6000 rpm, 25 minutes to make supernatant from *A. baumannii*. After centrifugation, the cells were precipitated at the lowest of the tube were rejected and supernatants were composed for use in AgNPs biosynthesis [9].

Molecular Identifications of Bacterial Isolates

Identification to species-level was determined by Polymerase Chain reaction (PCR) with universal primers, the 16S rDNA was subjected to nucleotide sequencing. Favor Prep Genomic DNA Mini Kit was used to extract total DNA following the manufacturer's protocol. The concentration of DNA and the purity of the DNA solution were determined by the spectrophotometer. PCR mixture 2.5 ul (10µM) 27F AGAGTTTGATCCTGGCTCA, 1492R GGTTACCTTGTTACGACTT, with Pre Mix- Kit, and 8µL of DNA sample, then completed to 20ul of DDW. PCR was run at 95 °C for 4 min then 38 cycles of 30 sec at 95 °C, 60 sec at 55 °C, and 120sec at 73 °C, 5min at 73°C. PCR product was performed electrophoresis on a one percent agarose gel with EB and photographed under UV then sequencing 16S rDNA57. The 16S rRNA gene sequences determined were aligned along with the sequences of type strains obtained from the GenBank by using the program CLUSTAL X (version 1.82) [60].

BioEdit (Sequence Alignment Editor version 4.7.8) (Hall, 1999) was used as the general tools for sequence editing and analysis e.g., for locating the putative ORFs in all reading frames, analyzing nucleotide composition, amino acids composition and codon usage ORF for performing multiple sequence alignment. The DNA sequence was subjected for similarity to the DNA sequences in NCBI, NLM, and NIH. The sequences used to achieve a BLASTN search against the sequence database [59].

Bacterial synthesis of AgNPs

$AgNO_3$ as substrate for bacterial synthesis of AgNPs by *A. baumannii.* Four concentration $AgNO_3$ (1,3, 5and 10mM) was added to *A. Baumannii* supernatant. Reaction condition was in dark room. The pH altered to 8. Then incubated at 36° C, 150 rpm for 18 hrs. The color change was noticed and the mixture was separated for 20 minute at 8000rpm, Supernatant was replaced with using centrifuged four times, the pellet dried at 45 °C for 30 hr.The powder was kept in vials for further tests [9, 13].

Characterization of AgNPs

XRD was used for structure and size of AgNPs, the powder of AgNPs was used for test. AFM was achieved for analysis the AgNPs the powder of AgNPs was used for the AFM. SEM (Inspect S50. FEI) was characterized to dimension, form and scattering of AgNPs [15].

Antibacterial action of AgNPs

Antibacterial actions of biological AgNPs carried with consuming agar well diffusion against some MDR bacteria of gram pos. and neg.(table 1). Tested bacteria with $1.5x10^8$cfu/mlinoculated onto MHAplates.Agar wells was achieved with 5 mm and 100µl from commercial AgNPs and AgNPs from *A. Baumannii* at concentration (150µg/ml) of AgNPs was added into each well. One petri dish sub cultured for each pathogenic bacterium and used as control and incubated for 18 hrs at 37°C[14].

Result

Molecular Identifications of Bacterial Isolates

The 16S rDNA sequence was considered as a more discerning (Chagnaud *et al*., 2001). Thus, 1270 bp of 16SrDNAwas amplified with PCR. From alignment with database in GenBank by BLAST program, bacteria were recognized with 99 % certainty to be *A. baumannii*.

Biosynthesis of AgNPs

Supernatant of *A .baumannii* demonstrated ability of synthesis AgNPs using $AgNO_3$(10mM) as an initiator for synthesis AgNPs and after shaking incubation for 18 hrs ,150rpm 38°C , *A. Baumannii* changed the color (figure 1) to reddish brown as pointer with creation AgNPs.

XRD categorization of AgNPs

The average size of silver nanoparticles was detected by X-ray crystallography diffraction (XRD) analysis, the *A. baumannii* produce silver nanoparticles with average size was 21 nm (figure 2).

AFM analysis of AgNPs

The average diameter and the three dimensional structure of silver nanoparticles was detected by the Atomic force microscope (AFM) analysis, the *A. baumannii* produce silver nanoparticles with average diameter 99.82nm (figure 3).Depending on description and characterization of nanoparticles by the color change, XRD and AFM. The morphology, size, distribution and presence of metals nanoparticles were characterized and a consequence .AgNO3 (10mM) was used for further study.

SEM categorization of AgNPs

SEM analysis exhibited well-isolated AgNPs, similar with diameter of 18-30nm, with inconstant shapes mostly spherical form (fig 4).

Antibacterial activity

Antibacterial activity of AgNPs(table 1)were biosynthesized by *A .baumannii* were used to estimate their ability in prevent growth of multidrug resistant bacteria(MDR).Agar well technique to identify the antibacterial activity of nanoparticles using commercial and biogenic AgNPs at concentration (150µg/ml) .After incubation the inhibition zone was measured in mm of the two types of AgNPs. The results specified that AgNPs inhibited the tested microorganisms (gram pos. and gram neg. bacteria). The inhibition diameter was bigger in gram neg. than in gram pos.(table2).The major inhibition zone of AgNPs was in Gram neg. bacteria with 15 mm in *Ps. aeruginosa* (7mg/ml) ,while the largest inhibition zone in Gram pos. bacteria was 16 mm in *S. pyogen*, while the biogenic AgNPs displayed large diameter in Gram negative bacteria which was 30 mm in *S. typhi* ;and large diameter zone in Gram positive bacteria was 23mm in *S. pyogen* with(7mg/ml) ,diverse antibacterial activity in further bacteria due to dissimilar in their sensitivity to AgNPs(table 2).

Table (2): Zone of inhibition of MDR bacteria in mm by AgNPs (7mg/ml).

microorganisms	Marketable AgNPs	AgNPs from *Aboumanii*
E.aerogenes	11	14
E.coli	14	20
K.pneumoniae	0	19
p. aeruginosa	15	23
P. agglomerans	0	16
P.mirabilis	0	15
S.aureus	0	15
S.pyogen	16	23
S.typhi	18	30

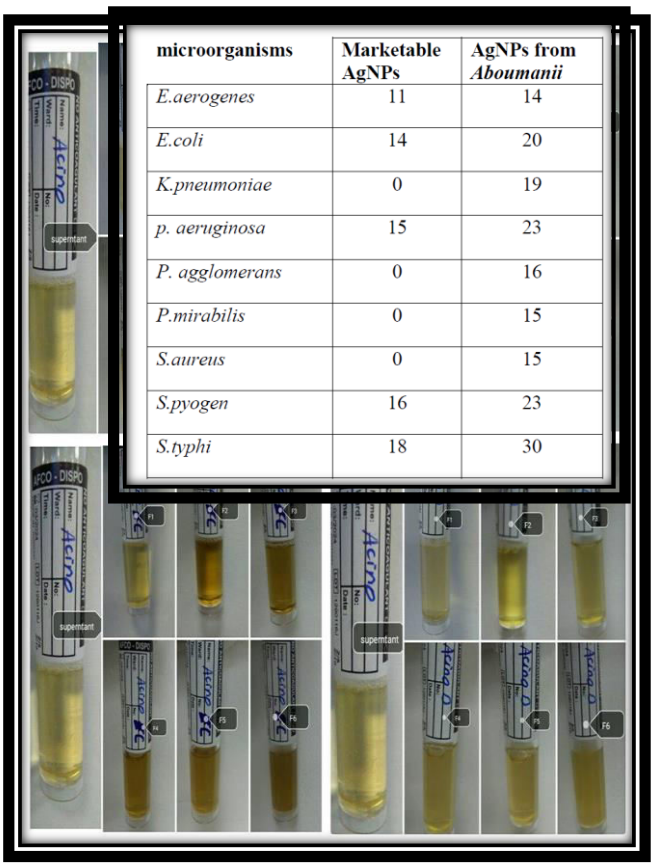


Figure (1): Optimization product for AgNPs using different concentration (1, 3, 5,10mM) for (2-48) hour's incubation time using *A. baumannii*. A-Concentration 1mM. B-Concentration 3mM C-Concentration 5mM D- Concentration 10mM. F1-Supernatant with $AgNO_3$before incubation

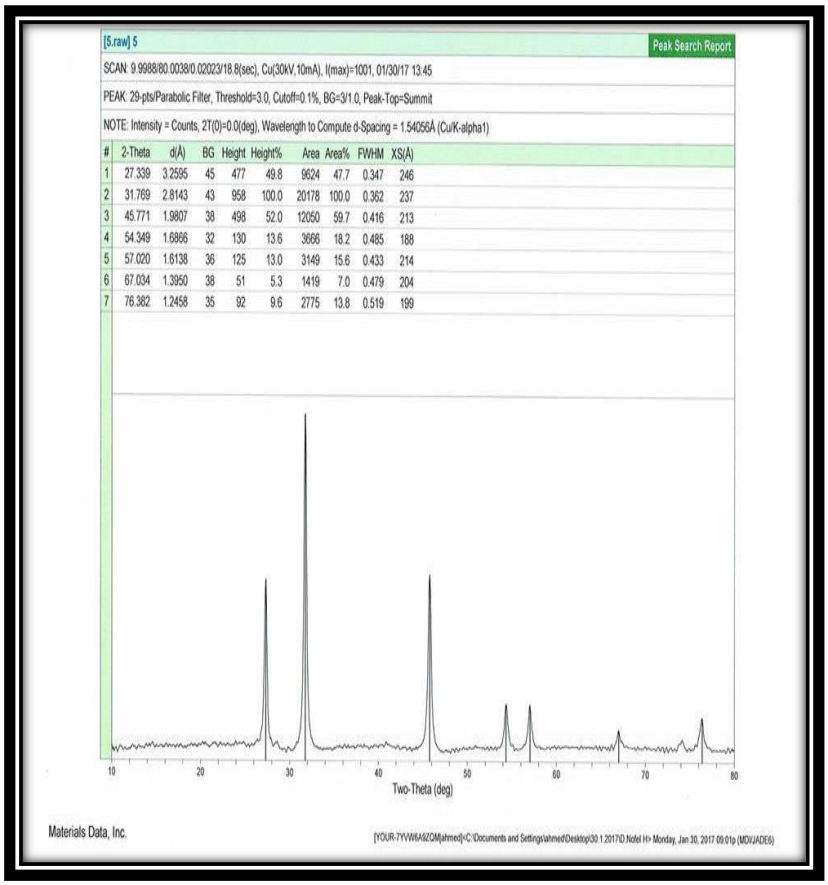
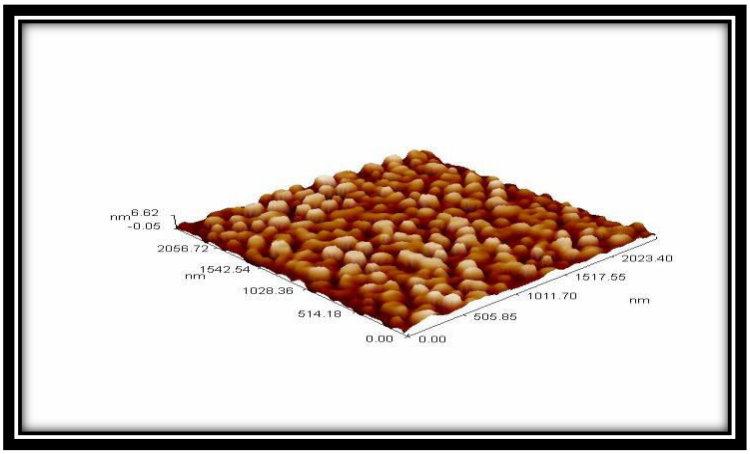


Figure (2): XRD analysis of Biosynthesized nanoparticles size from A.baumannii.



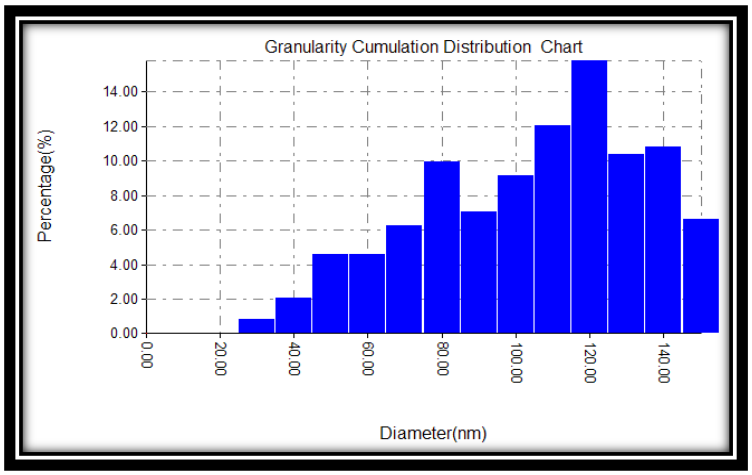


Figure (3): AFM analysis of synthesized AgNPs from *A. baumannii*. A-3D image of AgNPs. B-Scattering diagram of AgNPs

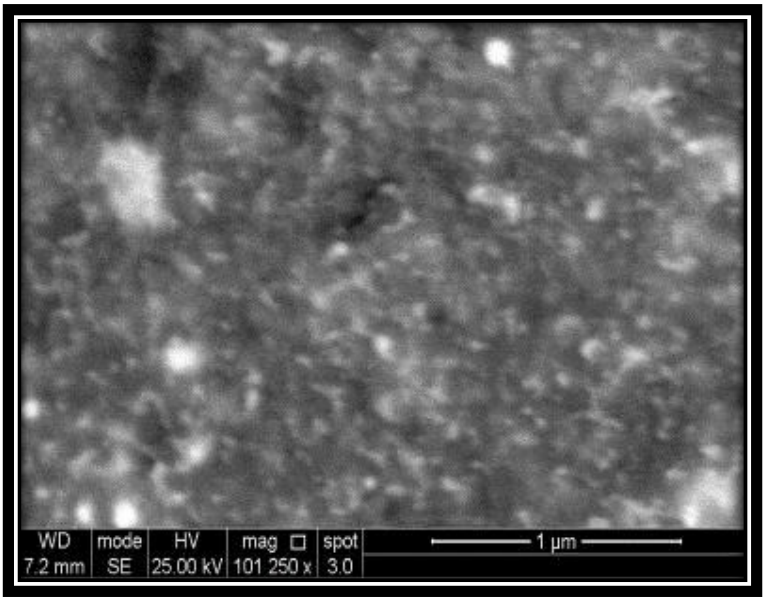


Figure (4): SEM analysis of synthesized AgNPs from *A.baumannii*

Discussion

AgNPs were biosynthesized with supernatant of *A. baumannii*. Morphological and biochemical characterization was confirmed as *A. baumannii*. When the reaction mixture containing supernatant of *A. baumannii* and different concentration of AgNO3 was added under dark conditions and adjustment of pH to 8.3, the medium color was changed from yellow to reddish-brown (fig 1). Presence of brown color is a vibrant suggestion of the synthesis of AgNPs as a reduction by the bacterial metabolites (enzymes, proteins, amino acid, polysaccharides, etc.) As reducing mediators in the supernatants [15, 16]. The color change is due to the coherent excitation of total electrons to surface plasmon resonance (SPR) [17].

Bacterial supernatant is the at ease way for the size-meticulous creation of AgNPs. The conditions of the supernatant can be effortlessly reformed and sustained than cells, components in the cytoplasm would attempt to preserve environment like heat shock proteins and require purity [18]. Not all the organisms are competent for the synthesis of silver nanoparticles; the exact reaction mechanism leading to the formation of silver nanoparticles by all organisms is yet to be elucidated. The organisms which comprise the "Silver resistance machinery" can create silver. Extracts from microorganisms may performance both as reducing and capping in AgNPs production. The reduction of silver ions by groupings of biomolecules [19].

Several studies designated that NADH- and NADH-dependent enzymes are significant factors in the creation of metal nanoparticles. The reduction appears to be originated by electron transfer from the NADH by NADH-

dependent reductase as electron carrier [20]. The Lactobacillus is notorious to yield nitrate reductase only overhead pH 6 which may be answerable for bioreduction of Ag+ to Ag0 and creation of AgNPs[21].

There is a likelihood that the aldehydic group existing in the extra polysaccharides secreted by the *Lactobacillus sp* might be elaborate in the reduction of silver ions to zerovalent silver (Ag 0)[22]. The form and dimension of nanoparticles based on physical with chemical aspects, e.g., reaction period, pH, composition reaction mixture and darkness [21]. NPS morphology can be regulated by synthesis with adjusting hydrogen ion concentration or heat of the mixture [23], also verified that at 64Cº fewer quantity of NPS were created, whereas at 36Cº extra quantity of nanoparticles was made. At acidic hydrogen ion concentration, the AgNPs production declined by reason of alkaline ion is necessary to the reduction of metal ions to less nucleation for Ag crystal development in fangled entering Ag atoms payment to make greater nanoparticles. While as the hydrogen ion concentration increase to alkaline, the dynamics of the ions and creation develop and extents to 10, additional nucleation areas are designed due to the obtainability of –OH ions. The transformation of Ag+ to Ag0 rises charted by a surge in the kinetics of the statement of the Ag atoms [24, 25].

Characterization of silver nanoparticles

SEM AgNPs synthesized using *A.baumannii* showed well dispersed, the diameter of 18-30nm with variable shapes mostly spherical form. The Characterization of biogenic nanoparticles was performed by Atomic force microscopy (AFM) and X-ray diffraction (XRD) analysis. The initial confirmation of the extracellular creation of AgNPs was achieved by the contrast colour change due to the surface Plasmon resonance (SPR) phenomenon. The conversion of extracellular moderate color evidently designates that the procedure of development of AgNPs is extracellular [26, 27]. The presence of brown color in a mixture encompassing the biomass is a strong suggestion of the creation of AgNPs in the reaction mix and is owing to the excitation of surface plasmon vibrations in the nanoparticles [28].

XRD noticed the middling size of AgNPs synthesized from *A.baumannii* was 21 nm (figure 2) the others indicated an average diameter of AgNPs from 20-100nm 29. AFM analysis detects the 3D structure of AgNPs and the average size of the nanoparticle was 99.82 nm belong to silver nanoparticle biosynthesized from *A.baumannii* (figure 3). After Characterization results (shape, size, and dispersity of nanoparticles), AgNPs were improved in inferiority; lowest size and few polydispersities, with *A.baumannii*, this could be indorsed to the alterations in the reduction that belong to extracellular metabolites, biomolecules that offered in the culture of microbes, furthermore, their capability of interaction with AgNO3 [30].

Antimicrobial activity of nanoparticles

Because of the emergence and increase in the number of multiple antibiotic-resistant microorganisms, nanoparticles are now considered alternate to antibiotics and have a great perspective to resolve this problematic. AgNPs were mostly good-looking for the making of a novel class of antimicrobials [31-33]. The AgNPs were tested to evaluate their antibacterial activity against gram-positive and gram-negative bacteria using the agar well diffusion method. The AgNPs looked at their actions on G+ve and G-ve bacteria (table2).

The biggest antimicrobials zone was displayed in bacteria, the extreme antimicrobials diameter of marketable AgNPs(150µg/ml) in gram-negative bacteria were 15mm with *Ps. aeruginosa* while 16mm in S. pyogenic. Biogenic AgNPs in gram-negative bacteria were 30mm in *S. typhi* and 23mm in gram-positive bacteria in *S. pyogen*. The difference between the peptidoglycan of gram-negative and gram-positive bacteria [34, 35, 36]. Variations in pathogens to AgNPs with 150µg/ml of marketable AgNPs such as *E.coli*, *Ps.aeruginosa* and *E.aerogenes*, the inhibition of these pathogens was 14,15 and 11 mm respectively, biosynthesized AgNPs from *A.baumannii* was 20,23,14 mm to the variances in susceptibility of pathogens based on several elements, intrinsic resistome[37]. AgNPs seems to be more lethal than Ag+ ions against *E. coli*[38- 40].

The positive charge is able to interact with the negative charge of bacteria with electrostatic action. Interaction can overpower other factors that can impact the bacterial death [41-43]. Silver ions could make structural changes in DNA and protein and cell wall. Ag ion interrelates with functional groups. The AgNPs injury membranes and discharge of ROS, making free radicals with antibacterial action [44, 45]. More small nanoparticles demonstrated more activity than big particles, due to a large surface area on microbe [46-53]. Hydrogen concentration has a characteristic role in the antimicrobial action with AgNPs, the lowest biogenic AgNPs in alkaline Hydrogen concentration exhibited more antimicrobial action than the big particles which are manufactured in acidic Hydrogen [54]. The shape of AgNPs shows very important in the antibacterial action of AgNPs. Hexagonal AgNPs display the maximum antimicrobial action related to further shape that was ascribed to the facet reactivity as well as particular surface areas [55, 56].

References

1. Bharathi, S.V. ,Ahmad,A. , Tajo,S.M. ;Silver Nanoparticle Synthesis Using C.Caesia and C.AmadaRhizome Extract ; International Journal of PharmTech Research;2015,Vol.8, No.7, pp 142-145.
2. Rai MK, Deshmukh SD, Ingle AP, Gade AK. Silver nanoparticles:thePowerfulnanoweapon against multidrug-resistantbacteria. J ApplMicrobiol. 2012; 112: 841e852.
3. Gajbhiye M, Kesharwani J, Ingle A, Gade A, Rai M. Fungusmediatedsynthesis of silver nanoparticles and their activity against pathogenic fungi in Combination with fluconazole.Nanomedicine 2009; 5: 382e386.
4. Kannaiyan, S.,Easwaramoorthi,Andal, V. ;Synthesis,Characterisation and Antibacterial activities ofSchiff base [New fuchsin] functionalised silver nanoparticles; International Journal of PharmTech Research ;2015,Vol.8, No.5, pp 54-60.
5. Murugan, M.,Shanmugasundaram,K.K.; Biosynthesis and characterization ofsilver nanoparticles using the aqueous of vitexNegundo,Linn;World Journal of Pharmacy and Pharmaceutical Sciences;2014 ,Volume 3, Issue 8, 1385-1393.
6. Lee PC, Meisel D. Adsorption and surface-enhanced Raman of dyes on Silver and gold sols. J PhysChem 1982; 86: 3391e3395.
7. Tanoue Y, Sugawa K, Yamamuro T, Akiyama T. Densely arranged two-dimensional silver nanoparticle assemblies with optical uniformity over vast areas as excellent surface-enhanced Raman scattering substrates. PhysChemChemPhys 2013; 15:15802e15805.
8. Mohanpuria, p. , Rana, N.K. , Yadav, S.K. ;Biosynthesis ofnanoparticles: technological concepts and future applications; Journal of Nanoparticle Research; 2008, vol. 10, no. 3, pp. 507–517.
9. Chaudhari, P.R. ,Masurkar, S.A. ,Shidore,V.B. ,Kamble,S.B. ; Antimicrobial activity of extracellularly synthesized silver nanoparticles using *Lactobacillus* species obtained from vizylac capsule; *Journal of Applied Pharmaceutical Science;* 2012,vol.02., no. 03,pp. 25-29.
10. Dhoondia,Z.H. ,Chakraborty,H. ; Lactobacillus Mediated SynthesisOf Silver Oxide Nanoparticles;Nanomater. nanotechnol.; 2012, Vol. 2, Art. 15:2012.
11. Karthick, S.,Namasivayam, R., Aroma,R. ,Manikanta, M. , Gopinath,P. ,.Francis ,A.L ; Evaluation of enzyme activity inhibition of biogenic silver nanoparticles against microbial extracellular enzymes ;*International Journal of PharmTech Research* ; 2016, Vol.9, No.2, pp 40-47.
12. Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T., Williams, S.T. Bergy,s Manual of Determinative Bacteriology. 9th.ed. Williams&Wilkins, 1994, USA: 532-551.
13. Sarvamangala, D. , Kondala, K. ,Murthy, U. S. N. ,NarasingaRao, B. Sharma, G. V. R. and Satyanarayana, R. ; Biogenic synthesis of AgNP's using Pomelo fruit—characterization and antimicrobial activity against Gram +Ve and Gram −Ve bacteria; *International Journal of Pharmaceutical Sciences* ;2013,vol.19,no. 2,pp.30–35.
14. Rajeshkumar, S., Malarkodi, C.; In Vitro Antibacterial Activity and Mechanism of Silver Nanoparticles against Foodborne Pathogens; *Bioinorganic Chemistry and Applications*; 2014, Volume 2014, 10 pages.
15. Natarajan, K.,Selvaraj, J. , Amachandra, V.; Microbial production of silver nanoparticles;*Digest Journal of Nanomaterials and Biostructures* ;2014,vol. 5,no. 1,pp 135 – 140.
16. Sreedevi, T.P., Thilagam, M., Tamil Selvi, A. AndChandrasekaran, B.; Synthesis,Characterization and AntibacterialStudies of Silver Nanoparticles Using *Lactobacillus Plantarum*; World*Journal of Pharmaceutical Research*;2015, vol.4,no. 8,pp1757-1773.
17. Gurunathan, S., Lee, K.J., Kalishwaralal, K., Sheikpranbabu, S., Vaidyanathan, R. and Eom, S.H.;Antiangiogenic properties of silver nanoparticles; *Biomaterials*; 2009, vol.3,pp 6341-6350.
18. Kalimuthu, K., Babu, R.S., Venkataraman, D., Mohd, B.andGurunathan, S. ; Biosynthesis of silver nanocrystals by *Bacillus licheniformis*; *Colloids Surf B Biointerfaces* ; 2008, 65(1):150-3.
19. Thakkar, K.N., Mhatre, S.S. , Parikh, R.Y.; Biological synthesis of metallic nanoparticles; Nanomedicine *nanotechnology biology and medicine*; 2010, vol.6, no.2, pp257-262.
20. Ranganath, E.,Rathod, V. ,Banu, A. ; Screening of Lactobacillus spp, for mediating the biosynthesis of silver nanoparticles from silver nitrate;*Journal of Pharmacy;* 2012, vol. 2,no.2,pp 237-241.
21. Durán, N., Marcato, P.D., Durán, M.,Yadav, A.,Gade, A. ,Rai, M.; Mechanistic aspects in the biogenic synthesis of extracellular metal nanoparticles by peptides, bacteria, fungi, and plants ;*Appl. Microbiol. Biotechnol.* 2011, vol. 90, pp 1609-1624.
22. Joerger R, Klaus T, Granqvist CG (2000) biologically produced silver carbon composite materials for optically functional thin-film coatings. Adv Mater 12:407–409.
23. Gericke, M. and Pinches, A., (2006). Biological synthesis of metal nanoparticles. J. Hydromet, 03.019, 83:132.
24. Gurunathan, S.**;** Lee, K.J.;Kalishwaralal, K.;Sheikpranbabu, S.;Vaidyanathan, R. and Eom, S.H. (2009).Antiangiogenic properties of silver nanoparticles. Biomaterials, 30: 6341-6350.

25. Sanghi, R.andVerma, P. (2009). Biomimetic synthesis and characterisation of protein capped silver nanoparticles. *BioresourTechno.l, 100*(1):501–504.
26. Bhainsa KC, D'Souza SF (2006) Extracellular biosynthesis of silver nanoparticles using the fungus Aspergillusfumigatus. Colloids Surf B 47:160–164.
27. Shaligram NS, Bule M, Bhambure R, Singhal RS, Singh SK, Szakacs G, Pandey A (2009) Biosynthesis of silver nanoparticles using aqueous extract from the compactin producing fungal strain. Process Biochem 44(8):939–943.
28. Ahmad, A.; Mukherjee, P.; Senapati, S.; Mandal, D.; Khan, M.I.; Kumar, R. and Sastry, M. (2003). Extracellular biosynthesis of silver nanoparticles using the fungus Fusariumoxysporum. Colloids Surfaces, B: Biointerfaces, 28 (4):313–318.
29. Abdulhassan,A.J.;(2016).Effect of Silver and Titanium Nanoparticles Synthesized by *Lactobacillus* as Antimicrobial ,Antioxidant and Some Physiological Parameters .Master Thesis .University of Kufa ,Faculty of Science – Iraq.:86-93.
30. Chaudhari, P.R.;Masurkar,S.A.; Shidore, V.B. and Kamble,S.P. (2012).Antimicrobial activity of extracellularly synthesized silver nanoparticles using *Lactobacillus* species obtained from vizylac capsule. *Journal of Applied Pharmaceutical Science,* 02 (03): 25-29.
31. Pinto, R.J.B.;Marques, P.A.A. ; Neto, C.P.; Trindade, T.;Daina, S. and Sadocco. P. (2009). Antibacterial activity ofnanocomposites of silver and bacterial or vegetable cellulo-sic fibers. *ActaBiomater.* 5: 2279-2289.
32. Lara, H.H.; Garza-Trevino, E.N.;Ixtepan-Turrent, L. and Singh, D.K. (2011).Silver nanoparticles are broad-spectrum bactericidal and virucidal compounds. *J. Nanobiotechnol,* 9: 30.
33. Rai, M.K.; Deshmukh, S.D.; Ingle, A.P. and Gade, A.K. (2012). Silver nanoparticles: The powerful nanoweapon against multidrug-resistant bacteria. *J. Appl. Microbiol*., 112: 841–852.
34. Kim, J.S.; Kuk,E.; Yu, K.N. ; Kim, J.H.; Park, S.J.; Lee, H.J.; Kim, S.H.; Park, Y.K.; Park, Y.H.(2007). Antimicrobial effects of silver nanoparticles. *Nanomed-Nanotechnol.*, 3: 9.
35. Thomas, J.;Silhavy, D.;Kahne, and Walker, S. (2010). The bacterial Cell Envelope. *Cold Spring HarbPerspect Biol.*, 2: 000414.
36. Taglietti, A.; Diaz Fernandez, YA. ;Amato, E. ; Cucca, L. ; Dacarro, G.;Grisoli, P.;Necchi, V. ; Pallavicini, P.; Pasotti, L. and Patrini, M. (2012).Antibacterial Activity of Glutathione-Coated Silver Nanoparticles against Gram Positive and Gram Negative Bacteria**.** *Langmuir*, 28 (21):8140–8148.
37. Blake, K. L. and O'Neill, A. J. (2013). Transposon library screening for identification of genetic loci participating in intrinsic susceptibility and acquired resistance to antistaphylococcal agents. *J. Antimicrob. Chemother*. 68(2):12–16.
38. Tian, J.,Wong, K. K. Y., Ho, C. M., Lok, C. N., Yu, W. Y., Che, C. M., Chiu, J. F. and Tam, P. K. H. (2006). Topical delivery of silver nanoparticles promotes wound healing. Chem. Med. Chem. 00: 171-180.
39. Shrivastava**, S**.;Bera, T.; Roy, A.; Singh, G.;Ramachandrarao, P.and Dash, D. (2007). Characterization of enhanced antibacterial effects of novel silver nanoparticles. *Nanotechnology*, 18: 225103–225112.
40. Soo-Hwan, K.; Lee, H.; Ryu, D.; Choi, S. and Lee, D. (2011). Antibacterial Activity of Silver-nanoparticles Against *Staphylococcus aureus and Escherichia coli*. *Korean J. Microbiol. Biotechnol.*, 39 (1): 77–85.
41. Fabrega, J. ; Fawcett, S.R.; Renshaw, J.C. and Lead, J.R. (2009). Silver nanoparticle impact on bacterial growth: effect of pH, concentration, and organic matter. *Environ Sci Technol.*, 43(19): 7285-7290.
42. Badawy, M.E. ; Silva, R.G. ; Morris, B. ; Scheckel, K.G. ; Suidan, M.T. and Tolaymat, T.M. (2011). Surface charge-dependent toxicity of silver nanoparticles. *Environmental Science and Technology*, 45 (1):283–287.
43. Abbaszadegan, A.; Ghahramani, Y.; Gholami, A.; Hemmateenejad , B.;Dorostkar, S.; Nabavizadeh, M. and Sharghi, H. (2015). The Effect of Charge at the Surface of Silver Nanoparticles on Antimicrobial Activity against Gram-Positive and Gram-Negative Bacteria. *Journal of Nanomaterials*, 3(4):8.
44. Wu, D. ;Fan, W. ; Kishen, A. ; Gutmann, J.L. and Fan, B. (2014). Evaluation of the antibacterial efficacy of silver nanoparticles against *Enterococcus faecalis*biofilm. *J. Endod.,* 40: 285–290.
45. Matsumura, Y.; Yoshikata, K.; Kunisak, S.andTsuchido, T. (2003). Mode of Bactericidal Action of Silver Zeolite and Its Comparison with That of Silver Nitrate. *Appl. Environ. Microbiol.,* 69: 4278–4281.
46. Vanaja, M. andAnnadurai, G. (2013). Coleus aromaticus leaf extract mediated synthesis of silver nanoparticles and its bactericidal activity. *Applied Nanoscience*, 3(1):217–223.
47. Chitra, K.andAnnadurai, G. (2014). Antibacterial Activity of pH-Dependent Biosynthesized Silver Nanoparticles against Clinical Pathogen. *BioMed Research International*, vol.2014, 6pages.

48. Wu, D.;Fan, W.;Kishen, A.;Gutmann, J.L. and Fan, B. (2014). Evaluation of the antibacterial efficacy of silver nanoparticles against *Enterococcus faecalis*biofilm. *J. Endod.,* 40: 285–290.
49. Lubick, N. (2008). Nanosilver toxicity: ions, nanoparticles- or both?. *Environ Sci& Technol.*, 42(23): 8617–8617.
50. Davod, T.; Reza, Z.; Ali, V.A. and Mehrdad, C. (2011).Effects of Nanosilver and NitroxinBiofertilizer on Yield andYield Components of Potato Minitubers. *Int. J. Agric. Biol.*, 13(6): 986–990.
51. Morones, J.R. ; Elechiguerra, J.L. ; Camacho, A. ; Holt, K. ; Kouri, J.B. ; Ramirez, J.T. and Yacaman, M.J. (2005). The bactericidal effect of silver nanoparticles. *Nanotechnology*, 16: 2346–2353.
52. Jung, W. K. ; Koo, H. C. ; Kim, K. W. ; Shin, S. ; Kim, S. H. and Park, Y. H. (2008). Antibacterial activity and mechanism of action of the silver ion in *Staphylococcus aureus*and*Escherichiacoli.Am. Soc. Microbiol.,* 74(7): 2171-2178.
53. Shrivastava ,S. ; Bera ,T.; Roy, A.; Singh, G. ; Ramachandrarao, P.and Dash, D. (2007). Characterization of enhanced antibacterial effects of novel silver nanoparticles. *Nanotechnology*, 18: 225103–225112.
54. Chitra, K.andAnnadurai, G. (2014). Antibacterial Activity of pH-Dependent Biosynthesized Silver Nanoparticles against Clinical Pathogen. *BioMed Research International*, vol.2014, 6pages.
55. El-Zahry, M.R. ;Mahmoud, A.; Refaat,I.H.; Mohamed, H.A.; Bohlmann, H. and Lendl, B. (2015).Antibacterial effect of various shapes of silver nanoparticles monitored by SERS. *Talanta*,138: 183–189.
56. Hong, X.; Wen, J.; Xiong,X. and Hu,Y. (2016).Shape effect on the antibacterial activity of silver nanoparticles synthesized via a microwave-assisted method. *Environmental Science and Pollution Research,* 23(5): 4489-4497.
57. Sambrook, J; and Rusell, D. W. (2001). Vectors and bacterial strains. Molecular Cloning, 3, A3.
58. Hall, T.A. (1999) BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT.
59. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25(17):3389-402.
60. Thompson JS, Ling X, Grunstein M.(1994).Histone H3 amino terminus is required for telomeric and silent mating locus repression in yeast. *Nature* 369(6477):245-7.