Journal homepage: http://fens.usv.ro/index.php/FENS
Journal of Faculty of Food Engineering,
Ştefan cel Mare University of Suceava, Romania
Volume XXI, Issue 2 - 2022, pag. 143 - 151

MICROBIAL SYNTHESIS OF SILVER NANOPARTICLES BY *ENTEROCOCCUS FAECALIS* STRAIN MI103

Jane NSOFOR[1], Imade Yolanda NSA[1], Busayo Tosin AKINYEMI[1], Adewale Kayode OGUNYEMI[1], *Matthew Olusoji ILORI[1]
[1]Department of Microbiology, University of Lagos, Nigeria
milori@unilag.edu.ng,
*Corresponding author:
Received 1st January 2022, accepted 25th June 2022

Abstract: Silver nanoparticles **(***AgNPs) have recently attracted attention for their potential as a source of new antimicrobials, with the potential to better fight a wide range of pathogenic bacteria. Nanomaterials are now commonly used for the production of industrial and domestic items. As a result of increase in the menace and threats to live by antibiotic-resistant bacteria, nanoparticles have been proposed as alternative for its control. Enterococcus faecalis, strain MI103, was selected out of four isolates obtained from a soil sample and identified using 16S rRNA gene sequencing method. The selected bacterial isolate synthesized AgNPs by reduction of Silver nitrate ($AgNO_3$) solution (incubated for 2 days at room temperature). Colour development from pale yellow to brown was observed which indicated the extracellular production of AgNPs. AgNPs was further characterized by UV/VIS spectroscopy, Scanning Electron Microscopy (JOEL-JSM-7660F) (shape) and Energy Dispersive X-ray (EDX) (composition). The synthesized AgNPs ranged in size of 90-100 nm and were of cylindrical shape. It was found that the highest quantity of $AgNO_3$ was produced at pH 7. The synthesized AgNPs had strong bactericidal activity against MRSA.*

Keywords*: Silver nanoparticles (AgNPs), Antimicrobials, Enterococcus faecalis, Nanotechnology, Nanoparticles, 16SrRNA*

1. Introduction

The development of antibiotic resistance has posed a significant threat, first to health and wellness and then to food security globally. As a result of increasing abuse of antibiotics in health and industrial use, antibiotic resistance has significantly increased [1, 2]. The development of antimicrobial drug resistance is making it increasingly difficult to treat otherwise routine infections effectively and this has created a pressing need for new antimicrobials. The costs associated with antibiotic resistance are higher, hospital stays are longer, and mortality increases [3]. Health care costs are rising due to antibiotic-resistant infections [4]. Antibiotics used without a prescription results in the emergence and spread of resistance. Actions must therefore be taken immediately to prevent infections and minor injuries from killing people around the world [5].

AgNPs have been proposed as an alternative to antibiotics in the race to curb the huge burden of infections that cannot be handled with the currently known antimicrobials. Nanoparticles are molecules with a minimum dimension of 100 nanometers [6]. In addition to chemical and biological sensing [7], CO_2 capturing [8, 9] and drug delivery [10], nanoparticles have found numerous applications. A number of

DOI: https://doi.org/10.4316/fens.2022.014

studies have shown that silver nanoparticles disrupt cell-walls and cellular respiration, as well as bind to them [11, 12, 13]. Several studies have shown that nanosilver is bactericidal against Gram-negative and Gram-positive bacteria. However, the underlying bactericidal mechanism is still unknown [14, 15, 16]. In a study by Morones *et al*. [15], four Gram negative bacteria, *E. coli*, *V. cholerae*, *P. aeruginosa* and *S. typhus*, were found to exhibit antibacterial activities using silver nanoparticles and had been shown to attach to the surface of the cell membrane, adhere bacteria, and discharge silver ions. There have been other studies with Gram positive bacteria, such as *Staphylococcus aureus* [15]. By examining the antibacterial properties of synthesized silver nanoparticles against drug-resistant bacteria, this study aims to determine the effectiveness of the biotechnolgicaly generated nanoparticles. Towards this end, methicillin-resistant *Staphylococcus aureus* (MRSA) was used.

2. Matherials and methods

2.1 Collection of Samples

Soil and water samples including loamy soil, oil contaminated sandy soil, seawater and tap water were collected in sterilized bottles from Lagos Mainland Local Government Area (LGA) of Lagos State and transported immediately to microbiological Laboratory for analysis.

2.2 Isolation of bacteria and biosynthesis of silver nanoparticles.

Bacteria was isolated by 10-fold serial dilution. About 0.2 ml aliquot were plated on Luria Bertani (LB) plate agar. Isolates were purified on LB agar medium in order to obtain pure cultures. The pure cultures were stored at 4°C on agar slant for further studies. The pure isolate was mass produced by cultivation on LB broth at 37°C for 24h on rotary shaker (150rpm). After incubation, cultures supernatants were obtained after centrifugation at 10,000 rpm for 10 min. One hundred millilitres of cell free supernatant were added to 50 ml of prepared 10 mM solution of $AgNO_3$ (1.7g/L). Three replicates were used for each strain. While cell free supernatant without $AgNO_3$ served as control [17]. The flasks were left for 24 h at room temperature in the dark to prevent photo catalytic action.

2.3 Characterization of Silver nanoparticles

Extracellular synthesized silver nanoparticles (AgNPs) produced by cell free cells supernatant of strains of isolated bacterial strains, obtained after centrifuging at 10,000 rpm for 10 minutes, were rinsed several times and dried. They were then characterized by UV/VIS spectroscopy in range of 200-700 nm [18]. The shape of the nanoparticles was determined by Scanning Electron Microscopy (JOEL-JSM-7660F) and the composition of the nanoparticles was determined by Energy Dispersive X-ray (EDX) [19].

2.4 Characterization of antibacterial activity of nanoparticles

Disc papers were soaked with 20 µL of 30 mg/L AgNPs suspensions and placed on the culture dish, incubated at 37°C for 24 h. For the control study, deionized water and generated Ag suspension soaked disc was also placed on the dish. These were then placed on Mueller Hilton agar that contained MRSA with approximate density of initial inoculum of about 0.5 Mc Farland turbidity standard. Sterilized cut filter papers that had been soaked in the cell-free water extract alone and $AgNO_3$ were used as control. Plates were incubated overnight at 35°C for 18 h and observed for the presence of zones of inhibition.

2.5 Identification of synthesizing bacteria

The bacteria isolate that produced the largest quantity of silver nanoparticles was identified using 16SrRNA gene sequencing method. Genomic DNA (gDNA) was extracted from the bacterial isolates using Zymo Research Bacterial DNA Miniprep^{tm} Kit (Zymo Research Corp USA). The DNA concentrations was determined using a NanoDrop 2000c spectrophotometer (ThermoFisher). Polymerase chain reaction (PCR) was used to amplify the 16S rRNA gene of the isolate using the primer pair 27F (5′-AGAGTTTGATCCTGGCTCAG-3) and 1492R 5'-GGTTACCTTGT TACGACTT-3) [20] was done in an Pielter thermal cycler (MJ Research Series) under the following conditions: initial denaturation step was 95°C for 5 minutes followed by 30 amplification cycles of 30 seconds at 95°C; 1 minute at 50°C and 1 minute 30 seconds at 72°C; and then a final extension step of 10 minutes at 72°C. The amplification product was separated on a 1.5% agarose gel and electrophoresis was carried out at 80 V for 1 hour 30 minutes. After electrophoresis, DNA bands were visualized by ethidium bromide staining. DNA sequences were processed by Basic Local Alignment Search Tool (BLAST) program (http://www.ncbi.nlm.nih.gov/BLAST).

2.6 Effect of pH on nanoparticle production and efficacy

The synthesizing AgNPs bacterial isolate was grown in LB broth at different pH 2, 7 and 9. The resulting suspensions were centrifuged and 100 ml of supernatant from each flask was inoculated with 50ml of 10mM of $AgNO_3$ and incubated in darkness. Production of nanoparticle was observed for in both flasks.

2.7 Test of AgNPs against Methicillin-resistant *Staphylococcus aureus* (MRSA)

Nanoparticles produced were tested against MRSA. Sterile filter papers were cut, soaked in the different nanoparticles produced and placed on plates already swabbed with an inoculum of MRSA and left to incubate overnight. Zones of inhibition were measured.

2.8 Evaluation of antibacterial activity, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of silver nanoparticles (AgNPs).

Kirby–Bauer Disk Diffusion Susceptibility Test method described by Bauer *et al.* [20] was employed for antibacterial activity of AgNPs against the MRSA pathogen. Sterile cotton swab was used for spreading the bacterial strain on Mueller-Hinton agar (MHA) (Merck, Germany). Sterile blank antimicrobial susceptibility disk was used in the test. The AgNPs concentration of 10mg/ml, 5mg/ml, 2mg/ml and 1mg/ml was loaded into the disks and was used for the test. The bacteria strain was spread on the Mueller-Hinton agar (MHA) (Merck, Germany) using sterile cotton swab. Sterile blank antimicrobial susceptibility disk was used in the test.

The antimicrobial efficacy determination by using method described by CLSI M07-A8. Here, observation for visible growth in the LB broth. The synthesized nanoparticles (4g) were suspended in 200 ml of LB broth using an ultrasonic homogenizer. The resulting (20 mg/ml) concentration was used as the stock concentrations. MIC was carried by inoculating 1ml of peptone water containing MRSA of approximate density of a 0.5 McFarland turbidity standard in all tubes. The tubes were incubated at 35°C for 24 hours and monitored for growth. The lowest concentration of silver nanoparticles where no visible growth is seen in the tubes is the MIC endpoint. However, the turbidity before and after the incubation was noted. MBC was carried out by inoculating 0.1ml from tubes that showed no visible growth (no turbidity) onto LB agar and incubating

for 24 hours at 35°C. MBC endpoint is the lowest concentration that is able to kill 99.9% of bacterial population. This was obtained by noting the pre- and post-incubated agar plates for the presence or absence of bacteria.

3. Results

3.1 Biosynthesis of silver nanoparticles

Among the four bacterial isolates, MI103 produced the highest quantity of brown precipitate (5g) indicating the presence of silver nanoparticles. One (one quarter) produced white precipitate and the other quarter of the (isolates) produced no precipitate. As a result of being the highest producer, MI103 was selected for further testing (Table 1). A colour change from pale yellow to brown was observed which was an indication of the extracellular synthesis of AgNPs. The precipitate was obtained by centrifugation at 10,000 rpm for 10 min and oven dried.

Table 1:
Screening for Production of Silver Nanoparticles

Organism code	Precipitate produced	Quantity
MI101	Brownish precipitate	0.8g
MI102	Whitish precipitate	0.5g
MI103	Brownish precipitate	5g
MI104	No precipitate	-

3.2 Characterisation of silver nanoparticles

Synthesized silver nanoparticles were characterized using UV-VIS spectrophotometry, scanning electron microscopy and Energy Dispersive X-ray (EDX). Figure 1 showed concentration of synthesized silver nanoparticles generated by isolate MI103 at different wavelengths. The absorbance obtained was within the range of 1.1 to 1.986 for synthesized silver nanoparticles. There was a steady rise in the absorbance readings between wave length of 200 nm to 300 nm, followed by a spike in absorbance readings at wavelengths between 380 nm and 426 nm until a decline occurred at 441 nm (Figure 1). Scanning electron micrograph (SEM) of synthesized silver nanoparticles showed that the particles were cylindrical in shape, highly clustered, dense and colloidal in nature (Figure 2). The nanoparticles are about 90-100 nm (verify size by measuring a particle calculating with 15nm scale bar) in size. Energy Dispersive X-ray (EDX) of silver nanoparticles showed that the particles contained several elements in the following concentrations: Silver (51.85%), Gold (2.70%), Oxygen (20.40%), Magnesium (2.44%), Sodium (5.90%), Silicon (3.75%), Calcium (2.26%) and Carbon (7.09%) (Figure 3).

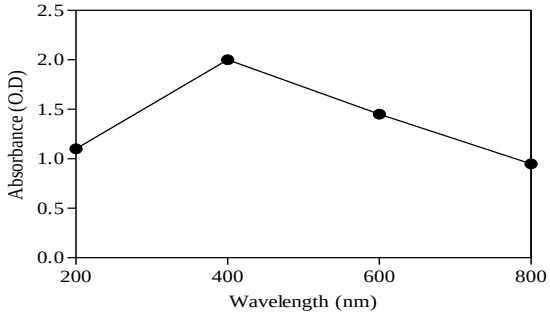

Fig. 1. Concentration of synthesized silver nanoparticles generated from MI103 at different wavelengths

3.3 Antibacterial efficacy, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of silver nanoparticles (AgNPs)

The antibacterial activity of AgNPs was determined against MRSA. The results for disk diffusion test, MIC and MBC of the AgNPs are summarized in Tables 2 and 3.

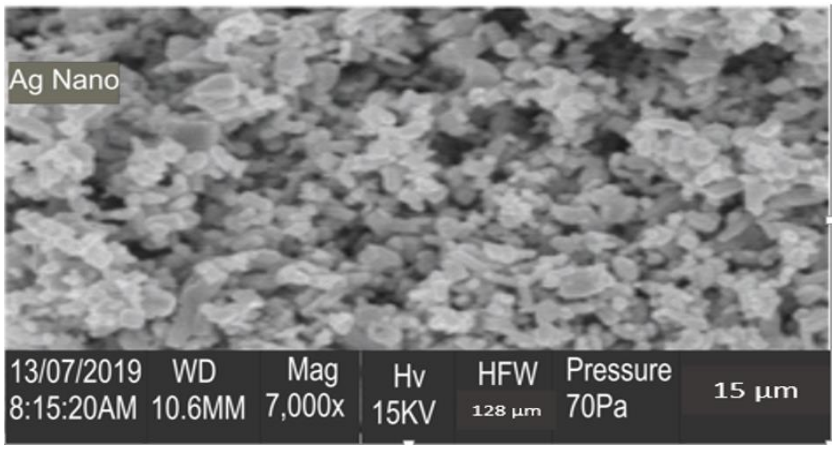

Fig. 2. Electron micrograph of silver nanoparticles generated from isolate MI103

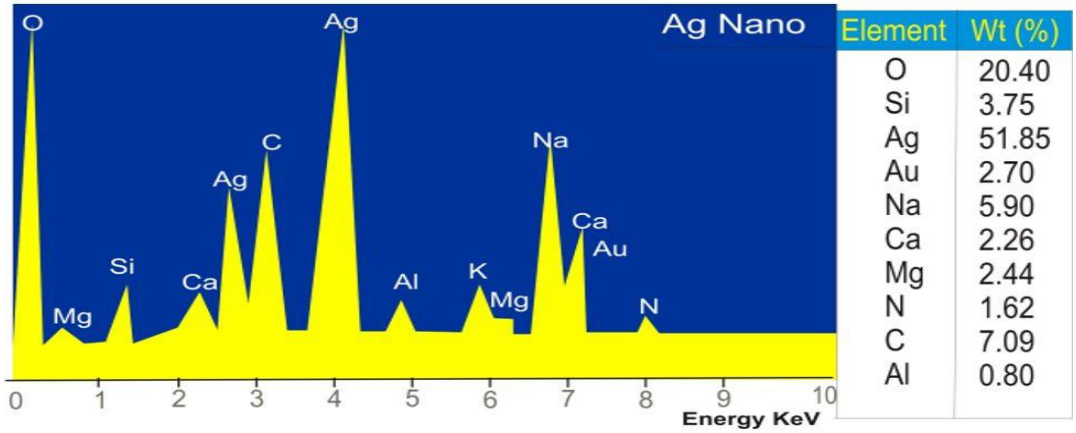

Fig. 3. Energy dispersive x-ray (EDX) of MI103 generated silver nanoparticles

For the disk diffusion test, the presence of clear zone around the AgNPs disk suggesting that the AgNPs possessed antibacterial activity which is able to inhibit the growth of the MRSA. The visible clear zone produced by AgNPs against MRSA bacteria is showed in Supplementary Plate 1. MRSA showed zones of inhibition ranging from 18 mm to

21 mm in diameter (Table 2). Thus, the antibacterial activity of Ag nanoparticles was bactericidal.

As showed in Table 3, MRSA showed MIC and MBC value of 5g/ml, respectively. In the study, the MIC and MBC value showed that MRSA was susceptible to AgNPs.

Table 2.

Antimicrobial (Antibacterial) Sensitivity Test (AST) of AgNPs against MRSA

Ag Nanoparticle Discs	1	2	3	4	5	6	7	8	9	10
Zone of inhibition against MRSA (nm)	18	21	21	18	20	19	20	18	20	18

Table 3.

Determination of MIC and MBC of AgNPs

MIC (mg/ml)	Observation	MBC (mg/ml)	Observation/any other description
20	Clear	20	No growth
10	Clear	10	No growth
5	Clear	5	No growth
2	Turbid	2	No growth
1	Turbid	1	No growth

Table 4.

Effect of pH on Production and Antimicrobial activity of AgNPs generated by MI103

pH	Effect on production		Effect on antimicrobial activity
	Growth in LB broth (after 24 hours)	Quantity of nanoparticles produced	Average zone of inhibition (mm)
2	Clear	-	-
7	Turbid	4.8g	25
9	Slightly Turbid	0.7g	10

3.4 Silver nanoparticle synthesizing bacteria

Isolate MI103, the highest producer of Ag nanoparticles, was identified as *Enterococcus faecalis* strain MI103 based on 16S rRNA gene sequencing method. The sequence (1506bp) was compared to sequences in GenBank by BLAST.

3.5 pH and nanoparticle production/activity

The optimal pH was obtained at 2 with no turbidity and precipitate observed in the LB after incubation in darkness. While at pH 7, the suspension was turbid and a brown precipitate formed. Whereas at pH 9, less turbidity and small mass (0.7 g) of precipitate formed (Table 3). MRSA were more sensitive to Ag nanoparticles at pH 7 than at pH of 9 by a ~ 2.5-fold difference in their zones of inhibition, 25 mm and 10 mm respectively (Table 3). The minimum inhibitory concentration of the synthesized nanoparticles was about 5 mg/ml (Table 4) as turbidity was observed at concentrations below the MIC indicating growth of MRSA. In addition, MBC of the synthesized nanoparticles is 5 mg/ml (Table 4) as no growth was observed after plating on LB agar from concentrations of 5 mg/ml and above which initially showed no turbidity.

4. Discussion

Research into alternative methods to cure infections has increased following reports of infections caused by antibiotic-resistant organisms. In this regard, nanoparticles have been used [21]. Because of their large surface area to volume ratio, silver nanoparticles have the most remarkable antimicrobial properties of all nanoparticles. Moreover, researchers are

interested in the growth of microbial resistance against metal ions, antibiotics, and the development of resistant strains [22, 23]. It is clear that these particles possess good antimicrobial properties; however, their exact mechanism of action remains unknown [24] plus the toxicological challenges that may result *in vivo*. There is evidence that AgNPs have action against a wide array of microbes due to the multiple mechanisms they utilize, including their ability to destroy drug-resistant bacteria, fungi, and viruses [25, 26, 27, 28, 29, 30]. It is true that there are various methods for synthesizing nanoparticles, but biological synthesis, which relies on plants or microbes, is environmentally and economically friendly [31]. A screen for microorganisms producing AgNP revealed *Enterococcus faecalis* to be the best candidate identified via the 16S rRNA gene sequence. Further characterization of biosynthesized AgNPs was achieved using UV-Vis spectroscopy and the peak was located between 420-440 nm [32]. There has been well documented evidence that this peak is caused by an interaction between two metal nanoparticles of different sizes ranging from 2 to 100 nanometers [33]. This study supports the findings of Jeevan *et al*. [17], where the peak was found between 420-430nm and [32], where the peak was at 420nm. It was reported [33] that extracts of *Parkia speciosa* Hassk pods were used in lieu of microbes, and the peak was found at 445 nm. SEM studies showed that the nanoparticles were cylindrical, dense, and clustered. Possibly, this is due to clumping and drying the samples prior to analysis. Similarly, Is [33] reports clumping. Compared to AgNPs with sizes ranging from 10 to 49 nanometers, these nanoparticles are 90-100 nm in size and irregular shape of synthesized by *Pseudomonas aeruginosa* (15-30nm) [34] and *Escherichia coli* (20-30nm) [35]. This study used Energy Dispersive X-ray (EDX) to examine AgNps which contained several elements, with silver being the most abundant. Several elements are present in AgNps, but silver is the most abundant, and the study examined it using Energy Dispersive X-ray (EDX). Is [33] came up with the same findings. It was found that AgNPs were extremely effective against methicillin - resistant *Staphylococcus aureus*. In previous studies, silver nanoparticles were tested for antimicrobial activity against methicillin-resistant *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Salmonella typhi* and *Klebsiella pneumonia* [35], *Escherichia coli* [17] and *Citrobacter* sp. [36]. As a result, nanoparticles were found to be effective in destroying them. In this study, it was found that nanoparticles were produced in the highest quantity at pH 7. In contrast, silver nanoparticles were not produced at pH 2 or 9 when pH was tested. There is evidence that the mechanism of producing nanoparticles is dependent on an enzyme, and enzymes are sensitive to pH fluctuations. In the synthesis carried out by Birla *et al*. [37], using *Fusarium oxysporum*, maximum nanoparticle production was observed between pH 9 and 11, with lower production occurring at pH 7 and aggregate formation occurring between pH 3 and 5. It has been noted, however, that Husseiny *et al*. [38] found a decrease in nanoparticle synthesis rate as pH increased, suggesting that reductases that are responsible for synthesis are less active at higher pH levels. The decrease in the efficacy of the nanoparticles produced against MRSA could also be attributed to the sensitivity to pH. The mode of antibacterial activity of the silver nanoparticles is thought to be bactericidal as there was no growth from zones of clearing indicating that the organisms had been killed as against the growth observed in the region where there was no clearing. The MIC and MBC of the AgNps were both found to be 5 mg/ml, which was similar to a

study by Krishnan *et al*. [39].

4. Conclusion

These results confirm that *Enterococcus faecalis* synthesized silver nanoparticles are bactericidal and are effective against antibiotic-resistant pathogens, thus providing a more efficient alternative to antibiotics.

5. Acknowledgments

The authors are indebted to Mr. James Ugah, a Technologist of Department of Microbiology, University of Lagos, Akoka, Lagos, Nigeria for his technical assistance during the course of the work. Also, we thank, Mr. Olanewaju Gabriel of the Electron Microscopy and Bio-Imaging laboratory, Mechanical Engineering of Covenant for his assistance in preparing the scanning electron micrographs of *Enterococcus faecalis* strain MI103.

6. References

[1]. UN (2001). WHO Global Strategy for Containment of Antimicrobial Resistance. Geneva, World Health Organization, 2001. http://www.who.int/drugresistance/WHO Global Strategy English.pdf.
[2]. UN (2005). WHO report, Antibacterial drug resistance options for concerted action. Norberg et al.archives.who.int/prioritymeds/.../antibacterial.do.
[3]. HOLMBERG SD.. SOLOMON SL.. BLAKE PA (1987) Health and economic impacts of antimicrobial resistance. Rev. Infect. Dis.9:1065-1078.
[4]. METZ M.. HLAES DM. (2014). Eight More Ways To Deal with Antibiotic Resistance Antimicrob Agents Chemother. 58(8): 4253–4256.
[5]. WHO (2018). Antibiotic resistance. Available at: http://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance. Last accessed: 9th July, 2018.
[6]. LAURENT S.. FORGE D.. PORT M.. ROCH A.. ROBIC C.. VANDER ELST L.. MULLER RN. (2010). Magnetic iron oxide nanoparticles: synthesis, stabilization, vectorization, physicochemical characterizations, and biological applications. Chem. Rev. 110: 2574.
[7]. BARRAK H.. SAIED T.. CHEVALLIER P, LAROCHE G, M'NIF A, HAMZAOUI AH (2016). Synthesis, characterization, and functionalization of ZnO nanoparticles by N-(trimethoxysilylpropyl) ethylenediamine triacetic acid (TMSEDTA): investigation of the interactions between phloroglucinol and ZnO. Arab. J. Chem. 882: 1–21
[8]. GANESH M.. HEMALATHA P.. PENG MM.. JANG HT. (2013) One pot synthesized Li, Zr doped porous silica nanoparticle for low temperature CO_2 adsorption. Arab. J. Chem. 41: 2740–2779
[9]. RAMACHARYULU PVRK.. MUHAMMAD R.. PRAVEEN KUMAR J.. PRASAD GK.. MOHANTY P. (2015). Iron phthalocyanine modified mesoporous titania nanoparticles for photocatalytic activity and CO2 capture applications. Phys. Chem. Chem. Phys. 17: 26456– 26462.
[10]. LEE JE.. LEE N.. KIM T.. KIM J.. HYEON T. (2011) Multifunctional mesoporous silica nanocomposite nanoparticles for theranostic applications. Acc. Chem. Res. 44: 893–902.
[11]. SINGH M.. SINGH S.. PRASAD S.. GAMBHIR IS. (2008) Nanotechnology in medicine and antibacterial effect of silver nanoparticles. Dig. J. Nanomater. Bios. 3: 115-122.
[12]. DURÁNA N.. DURÁNB M.. BISPO DE JESUS M.. SEABRAF AB.. FÁVAROB WJ.. NAKAZATOG G. (2016). Silver Nanoparticles. A New View on Mechanistic Aspects on Antimicrobial Activity Nanomedicine 12(3):789-799.
[13]. HAMOUDA RA.. HUSSEIN MH.. ABO-ELMAGD RA.. BAWAZIR SS. (2019). Synthesis and biological characterization of silver nanoparticles derived from the cyanobacterium *Oscillatoria limnetica*. Sci Rep 9:13071https://doi.org/10.1038/s41598-019-49444-y
[14]. WU D.. FAN W... KISHEN A.. GUTMANN JL.. FAN B. (2014). Evaluation of the antibacterial efficacy of silver nanoparticles against Enterococcus faecalis biofilm. J Endod 40:285-90.
[15]. MORONES JR.. ELECHIGUERRA J.. CAMACHO A.. HOLT K.. KOURI JB.. TAPIA J.. Yacaman MJ. (2005). The bactericidal effect of silver nanoparticles. Nanotechnology 16:2346–2353
[16]. SHRIVASTAVA S.. BERA T.. ROY A.. SINGH G.. RAMACHANDRARAO P.. DASH D. (2007). Characterization of enhanced antibacterial effects of novel silver nanoparticles. Nanotechnology 18:1–9
[17]. JEEVAN P.. RAMYA K.. RENA AE. (2012). Extracellular biosynthesis of silver nanoparticles by culture supernatant of *Pseudomonas aeruginosa*. Indian J. Biotechnol. 11: 72-76.
[18]. ABO-STATE M.. PARTILA A. (2015). Microbial Production of Silver Nanoparticles by

Pseudomonas aeruginosa Cell Free Extract. J. Eco. Heal. Env. 3(3): 91-98
[19]. AYESHA A. (2017). Bacterial Synthesis and Applications of Nanoparticles. Nano. Sci. Nano. Technol. 11(2):119
[20]. BAUER AW.. KIRBY WM.. SHERRIS JC.. TURCK M. (1966). Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 45:493–496..
[21]. VALLET-REGÍ M.. GONZÁLEZ ISABEL. ZQUIERDO-BARBA B. (2019). Nanomaterials as Promising Alternative in the Infection Treatment. Int. J. Mol Sci. 20(15): 3806. doi: 10.3390/ijms20153806
[22]. DURÁN N.. MARCATO PRISCYLA D… DE CONTI ROSELI ALVES OSWALDO L. COSTAB FTM.. BROCCHIB M, (2010), Potential use of silver nanoparticles on pathogenic bacteria, their toxicity and possible mechanisms of action. J Braz Chem Soc 21(6):949–959
[23]. KEAT CL.. AZIZ A.. EID AM.. ELMARZUGI NA. (2015). Biosynthesis of nanoparticles and silver nanoparticles. Bioresour Bioprocess 2:47
[24]. PELGRIFT RY.. FRIEDMAN AJ. (2013). Nanotechnology as a therapeutic tool to combat microbial resistance. Adv. Drug Deliv. Rev. 65:1803–1815.
[25]. SONDI I.. SALOPEK-SONDI B. (2004). Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria. J. Colloid Interface Sci. 275:177–182. doi: 10.1016/j.jcis.2004.02.012
[26]. PAL S.. TAK YK.. SONG JM. (2007). Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the Gram-negative bacterium *Escherichia coli*. Appl. Environ. Microbiol. 73:1712–1720. doi: 10.1128/AEM.02218-06
[27]. FAYAZ M.. BALAJI K.. KALAICHELVAN PT.. VENKATESAN R. (2009). Fungal based synthesis of silver nanoparticles—an effect of temperature on the size of particles. Colloids Surf. B. Biointerfaces 74:123–126
[28]. LARA HH.. AYALA-NÚNEZ NV.. DEL CARMEN IXTEPAN TURRENT L, PADILLA CR (2010). Bactericidal effect of silver nanoparticles against multidrug-resistant bacteria. World J. Microbiol. Biotechnol. 26:615–621. doi: 10.1007/s11274-009-0211-3
[29]. SAMBERG ME.. ORNDORFF PE.. MONTEIRO-RIVIERE NA (2011). Antibacterial efficacy of silver nanoparticles of different sizes, surface conditions and synthesis methods. Nanotoxicology 5:244–253. doi: 10.3109/17435390.2010.525669
[30]. YOUJ ZHANG Y.. HU Z. (2011). Bacteria and bacteriophage inactivation by silver and zinc oxide nanoparticles. Colloids Surf. B Biointerfaces 85:161–167. doi: 10.1016/j.colsurfb.2011.02.023
[31]. TILLMANN P. (2004) Stability of silver nanoparticles in aqueous and organic media. J. Matter. Chem. 4:140-146.
[32]. QUINTEROS MA.. AIASSA IM.. MARTÍNEZ IM.. DALMASSO PR.. PÁEZ PL. (2016). Silver Nanoparticles: Biosynthesis Using an ATCC Reference Strain of *Pseudomonas aeruginosa* and Activity as Broad Spectrum Clinical Antibacterial Agents. Int. J. Biomater. 2016:1-7
[33]. IS F (2016). Green synthesis of silver nanoparticles using extract of Parkia speciosa Hassk pods assisted by microwave irradiation. J. Adv. Res. 7:961-969
[34]. DU L.. JIANG H.. LIU X.. WANG E. (2007). Biosynthesis of gold nanoparticles assisted by *Escherichia coli* DH5α and its application on direct electrochemistry of haemoglobin. Electrochem. Comm. 9(5):1165–1170.
[35]. NANDA A.. SARAVANAN M. (2009). Biosynthesis of silver nanoparticles from *Staphylococcus aureus* and its antimicrobial activity against MRSA and MRSE. Nanomedicine 5: 452–456.
[36]. PRIYA MM.. SELVIA BK.. PAUL JA. (2011). Green synthesis of silver nanoparticles from the leaf extracts of *Euphorbia hirta* and *Nerium indicum*. Dig. J. Nanomater. Bios. 6: 869 – 877.
[37]. BIRLA SS.. GAIKWAD SC.. GADE AK.. RAI MK.. (2013). Rapid Synthesis of silver nanoparticles from *Fusarium oxysporum* by optimizing physicocultural conditions. Sci. World J. 2013:1–12.
[38]. HUSSEINY SM.. SALAH TA.. ANTER HA. (2015). Biosynthesis of size controlled silver nanoparticles by *Fusarium oxysporum*, their antibacterial and antitumoral activities. Beni Suef Univer. J. Basic Appl. Sci. 4:225–31. doi: 10.1016/j.bjbas.2015.07.004.
[39]. KRISHNAN R.. ARUMUGAM V.. VASAVIAH SK. (2015). The MIC and MBC of Silver Nanoparticles against *Enterococcus faecalis* - A Facultative Anaerobe. J. Nanomed. Nanotechnol. 6: 285.