International Journal of Modern Agriculture, Volume 10, No.2, 2021
ISSN: 2305-7246

Antimicrobial activity of silver nano-particles synthesized from Bacillus tequilensis

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Abstract

Enteric diseases are considered as the most prevalent health related issues. Antibiotics are used to combat these diseases in a variety of ways. Numerous bacteria have developed resistance to these antibiotics, which is a major problem in health sector. Antibiotic resistance has prompted scientists to create new techniques. *Bacillus tequilensis* is a kind of bacterium that can help in synthesis of biological nanoparticles. The main objective of our study was to synthesize silver nanoparticles from *Bacillus tequilensis* and then to determine its antimicrobial activity. It was first proven by the color shift. Well diffusion method was used to test antibacterial activity. Muller Hinton agar medium was used to grow the strains. In contrast to *Bacillus cereus, Escherichia coli* showed a high vulnerability to these nanoparticles in antimicrobial assays. With regards to *Escherichia coli*, the maximum zone of inhibition was 15-25mm, whereas the lowest was 6-9mm against *Bacillus cereus.* Our study concludes that *Bacillus tequilensis* can be used to synthesize nanoparticles. It is possible to synthesize nanoparticles on large industrial levels with a wide range of potential uses.

Key words: Antimicrobial activity; Silver nano-particles; enteric diseases; *Bacillus tequilensis*

Introduction

Infections in human are caused by different pathogens. Antibiotic resistance in acquired by these pathogens has been a growing problem in recent years (1). The overuse of antibiotics in human and veterinary medicine has led to a rise in drug resistance. Antibiotic resistance is frequent in bacteria such as *Mycobacterium TB, Acinetobacter baumannii, Klebsiella spp., Escherichia coli, Enterococcus spp* and *Streptococcus pneumonia* (2). Antibiotic resistance is a global problem, and nanomaterials offer an innovative way to combat it. They circumvent resistance by penetrating the cell wall of the bacterium against which they are being employed as an antibacterial agent (3).

Multidrug resistance in human infections is one of the most serious issues, and finding an alternative to antibiotics is essential to solving the problem. AgNPs are an alternative natural source that has the capacity to manage disease resistance. The high surface-to-volume ratio and crystalline shape of these particles make them promising antibacterial agents (4).

One of the most important areas of nanotechnology is the advancement of biological nanoparticle production. They have a bactericidal and inhibitory activity that is consistent. They are effective against

the majority of multi-resistant bacteria (5). The technique of killing bacteria reveals that silver ions enter the bacterium's cell and take over the genomic molecules, shrinking the DNA molecule and, as a result, the cell's capacity to replicate is lost, and the cell remains non functional, leading to cell death (6). Silver nanoparticles may be synthesized, which has just recently been described. It has also been shown that AgNPs may be synthesized extracellularly by combining the culture supernatant of B. subtilis species and microwave irradiation in water. Silver nanoparticles may also be produced by *K. pneumoniae, Enterobacter cloacae* and *E. coli*. The use of *B. megaterium* cultured supernatant has also been reported to form extracellular particles in water solution and silver ions in a short period of time. The biomolecules of the cell decrease the metal ions when they are linked to the outer membrane, results in the formation of nanomaterials. In addition, recently isolated *Bacillus tequilensis* CDW-64 from contaminated soil has been shown to exhibit fibrinolytic activity. *Bacillus tequilensis* CDW-64 is a Gram-positive, spore-forming bacteria with the potential to create extracellular and intracellular enzymes (7) .

Antibacterial agents such as enzymes, antibiotics, ions, metals and ammonium compounds are widely used in daily life to protect the public's health. In addition to these detrimental consequences on human health, these materials are also harmful to the environment and expensive, making them unsuitable for human consumption. As a result, innovative, low-cost, and environmentally friendly antibacterial agents are required. Using novel biological techniques, this research aimed to find low-cost, environmentally friendly, and nontoxic approaches to synthesize AgNPs.

Materials and methods

Area of study

This research was carried out in the NIGAB department of the National Agriculture Research Centre Islamabad's probiotic development research laboratory.

Sample collection

The department provided all specified samples, including Bacillus bacterial and pathogenic strains. The samples were maintained in the freezer until they were ready to be processed.

Culture of bacteria

By using a sterile wire loop to remove the colony from previously given plates, the identified culture was refreshed. Solid MRS Agar medium sterile plates were used to streak the colonies and Parafilm was used to cover the plate. In the incubator, the cultivated plates were inverted and incubated for 24 hours at 37°C. Growth was subcultured after 24 hours to get pure colonies of identified *B. tequilensis*.

Identification of bacteria

All the bacteria were identified by colony morphology, gram staining and biochemical tests.

Preparation of AgNps

We used sterilized LB broth in Erlenmeyer flasks. With the use of a pipette tip, a colony was selected and mixed into the LB broth. In a shaker incubator, the flask was incubated at 37°C for 24 hours. The broth's turbidity was determined after 24 hours. Later, the broth was centrifuged for ten minutes at 10000rpm. A syringe filter was used to collect the supernatant. A 5mM AgNO3 solution was then added to the sample. In the incubator, the solution was maintained at 60°C for 1 hour. The color of the solution

was examined after 1 hour. Due to surface Plasmon resonance, the sample's color shifted from yellow to brown, indicating that silver nanoparticles had been synthesized.

Characterization Technique

UV-Vis **spectra analysis**

First, a hollow quartz cuvette with a route length of 1 cm was taken and examined in the laboratory. The production of silver nanoparticles was then validated by measuring the wavelength of the solution in a spectrophotometer with a resolution of 1nm from 200 to 800nm with an observing rate. At 422nm, the highest peak was seen. According to a survey of the literature, it was discovered that the peak of silver nanoparticles occurs between 420 and 430nm in diameter (8).

Anti-Microbial Activity

The outcomes of the nanoparticles were examined using the agar well diffusion technique against the clinical pathogenic pathogens *B. cereus, S. aureus, E. coli,* and *L. monocytogene*. Using the use of a cotton swab, the aforementioned microorganisms were inoculated into MHA medium, and 5mm holes were drilled with pipette tips. Agarose was put to the holes to cover the bottoms and prevent the material from dispersing. After that, a 5 microliter sample was added to each well, as well as a negative control well with no sample. There was also a positive control used. At 37°C, the plates were incubated for 24 hours. The zones of inhibition were examined after 24 hours. Zones of inhibition were seen at various volumes of samples.

Results

On the MRS plates, *B. tequilensis* appeared as congested colonies (Figure.1). Microscopy revealed that these bacteria were Gram-positive rods. *B. tequilensis* tested positive for catalase and oxidase. The reduction of silver ions into silver particles was noticed by changing the color of the solution from clear yellow to dark brown after adding the AgNO3 solution to the bacterial supernatants. The color of the supernatant and AgNO3 was yellow in Figure 2(a), but after 1 hour in the incubator at 60°C, the color changed to brown, as shown in Figure 2. (b). The solution's brown color indicated that the silver nanoparticles had been produced. UV spectrophotometer analysis was utilized for further validation. Between the wavelengths of 420 and 430 nm, narrow and sharp bands were noticed. The peak of the SPR will be up to 422 nm, according to various evaluations and literature. As a result, the production of silver nanoparticles has been verified. With the use of a foot meter, zero zones of inhibition were detected. All of the strains were resistant to most antibiotics, with the exception of a few that were susceptible, as indicated in Table 1.

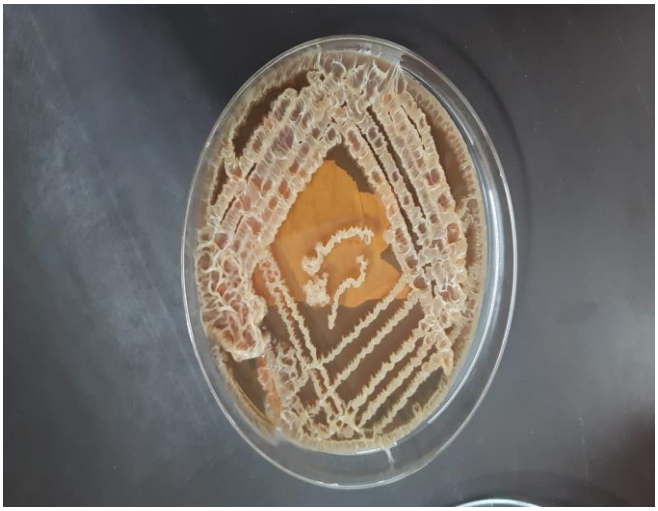

Figure 1: growth of *B. tequilensis* on MRS

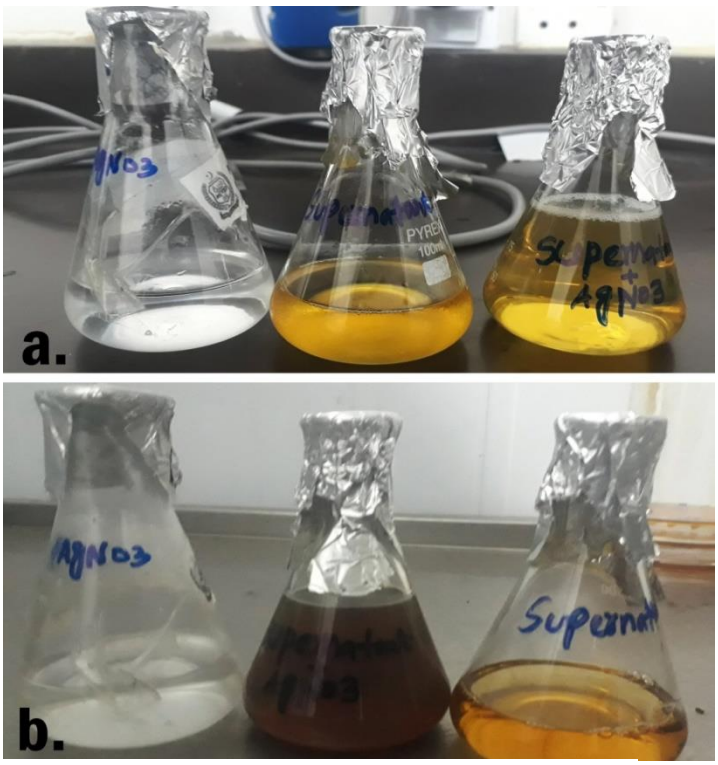

Figure 2: Silver nanoparticles confirmation from color change

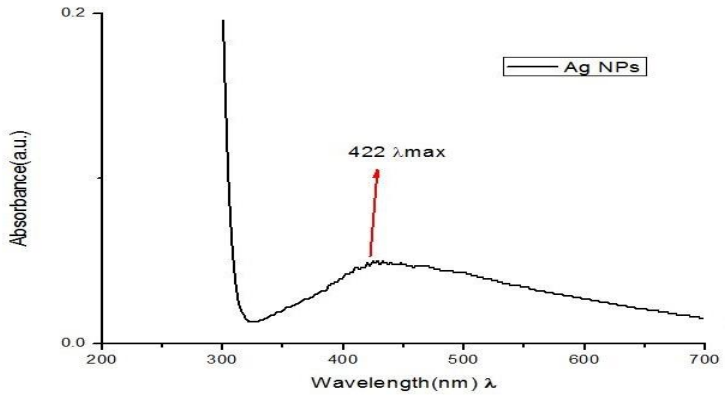

Figure Error! No text of specified style in document.**: Silver nanoparticles analysis by using UV VIS-Spectrophotometry**

Table 1: Antibiogram assay of the selected bacteria

Antibiotic used	**Name of the resistant isolates**			
	E. Coli	***B. cereus***	***L. monocytogenes***	***S. aureus***
Cephalothin	Resistance	Resistance	Resistance	Resistance
Amoxicillin	Resistance	Resistance	Resistance	Resistance
Cefoperazone	Sensative	Resistance	Resistance	Sensative
Ceftriaxone	Resistance	Resistance	Resistance	Sensative

Silver nanoparticles synthesized from *B. tequilensis* were tested against several harmful bacterial strains for antibacterial efficacy. In contrast to *Bacillus cereus, Escherichia coli* showed a high vulnerability to these nanoparticles in antimicrobial assays. With regards to *Escherichia coli,* the maximum zone of inhibition was 15-25mm, whereas the lowest was 6-9mm against *Bacillus* ***cereus.*** **(**Table 2)

Table 2: Antimicrobial activity of silver nanoparticles against selected bacteria

Bacterial strains	**NC**	**PC $AgNO_3$ solution(15µl)**	**10µl**	**15 µl**	**25 µl**
S. aureus	--	7-11mm	12-15mm	13-17mm	19-20mm
L. monocytogenes	--	6-9mm	11-13mm	14-16mm	17-19mm
B. cereus	--	7-9mm	10-12mm	13-15mm	16-18mm
E. coli	--	13-15mm	16-18mm	20-23mm	22-25mm

Discussion

Silver nanoparticles were synthesized in this study by mixing AgNO3 with bacterial culture supernatants. Colour change indicated the reduction of Ag ions from AgNO3 to AgNPs. The AgNO3

and bacterial supernatant combination becomes brown when exposed to Surface Plasma Resonance. The previous work used Bacillus clausii cultivated from enterogermina to make biogenic silver nanoparticles that show a strong color shift owing to Plasmon resonance absorption. UV spectrophotometry analysis was used to confirm the formation of silver nanoparticles (9). Silver nanoparticles were synthesized in another study by mixing of silver nitrate and *fusarium oxysporum* mycelia. A change in color from yellow to brown indicated the presence of silver nanoparticles. Because of their surface, spectrophotometers were used to confirm SNP generation at maximal absorbance peaks of about 420-430nm. Plasmon resonant frequency (10).

The SPR peak at 422nm, which is comparable to earlier consulted research, was used to confirm AgNPs synthesis in this work (11). Ag+ ions and silver-based compounds, such as AgNPs, exhibit apparent antibacterial properties against multidrug-resistant organisms (12). Four distinct bacteria were chosen as representative species to test the antibacterial impact of AgNPs. A well diffusion experiment was used to test AgNPs' antimicrobial properties (13).

These silver nanoparticles were shown to exhibit antibacterial properties against all of the microorganisms tested in the well diffusion assay. The size of the zone of inhibition and the concentration of AgNPs against all microorganisms had a direct relationship. All other bacteria utilized in this research were less susceptible to these nanoparticles than E. coli. Even at low concentrations (10ul), E. coli displayed a wide zone of inhibition (15-19mm). These Biogenic AgNPs were equally potent to *Listeria* and *S. aureus.* At the lowest dose (10ul), both demonstrated a zone of inhibition of 9-11mm that rises to 18-19mm as concentration increases (25ul). In comparison to the other bacteria utilized in this experiment, *B. cereus* was less sensitive to these nanoparticles. B. cereus displayed a zone of inhibition of 6-9mm at a concentration (10ul) and grew to 16mm at 25ul. There was no zone of inhibition surrounding the control wells that were not diluted with AgNP. Previous study reported similar results to our study (14). In this study, several concentrations of AgNPs were employed, ranging from 15ul to 25ul. The size of the inhibitory zone is proportional to the concentration of AgNPs. AgNPs have a stronger antibacterial impact against Gram-negative bacteria than Gram-positive bacteria, according to our findings. This might be due to different cell wall structures. As a result, AgNP adhesion to microbial surfaces varies. The presence of a thick peptidoglycan layer on Gram-positive bacteria's outermost boundary may prevent silver nanoparticles from adhering to the bacterial surface. We observed that E. coli was more susceptible to silver nanoparticles than *S. aureus* which is consistent with another research that employed *E. coli, S. aureus,* and yeast as representative species to investigate antibacterial activity of silver nanoparticles and reported that *E. coli* was more sensitive to silver nanoparticles than *S. aureus* and yeast (15).

Conclusion

It is commonly known that the majority of harmful bacteria are antibiotic resistant. As a result, other ways for controlling multidrug-resistant pathogens that are less expensive and time-consuming are required. This research used a simple approach to develop silver nanoparticles that were antimicrobial. This research indicates that employing *B. tequilensis* to synthesize silver nanoparticles is a simple and efficient strategy.

REFERENCES

1. Cavassin ED, de Figueiredo LFP, Otoch JP, Seckler MM, de Oliveira RA, Franco FF, et al. Comparison of methods to detect the in vitro activity of silver nanoparticles (AgNP) against multidrug resistant bacteria. Journal of nanobiotechnology. 2015;13(1):64.

2. Qais FA, Shafiq A, Khan HM, Husain FM, Khan RA, Alenazi B, et al. Antibacterial Effect of Silver Nanoparticles Synthesized Using Murraya koenigii (L.) against Multidrug-Resistant Pathogens. Bioinorganic chemistry and applications. 2019;2019.

3. Fernando S, Gunasekara T, Holton J. Antimicrobial Nanoparticles: applications and mechanisms of action. Sri Lankan Journal of Infectious Diseases. 2018;8(1):2-11.

4. Mitiku AA, Yilma B. Antibacterial and antioxidant activity of silver nanoparticles synthesized using aqueous extract of Moringa stenopetala leaves. African Journal of Biotechnology. 2017;16(32):1705-16.

5. Fayaz AM, Balaji K, Girilal M, Yadav R, Kalaichelvan PT, Venketesan R. Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: a study against gram-positive and gram-negative bacteria. Nanomedicine: Nanotechnology, Biology and Medicine. 2010;6(1):103-9.

6. Yan X, He B, Liu L, Qu G, Shi J, Hu L, et al. Antibacterial mechanism of silver nanoparticles in Pseudomonas aeruginosa: proteomics approach. Metallomics. 2018;10(4):557-64.

7. Velusamy P, Pachaiappan R, Christopher M, Vaseeharan B, Anbu P, So J-S. Isolation and identification of a novel fibrinolytic Bacillus tequilensis CWD-67 from dumping soils enriched with poultry wastes. The Journal of general and applied microbiology. 2015;61(6):241-7.

8. Kushwaha A, Singh VK, Bhartariya J, Singh P, Yasmeen K. Isolation and identification of E. coli bacteria for the synthesis of silver nanoparticles: characterization of the particles and study of antibacterial activity. Eur J Exp Biol. 2015;5(1):65-70.

9. Mukherjee K, Gupta R, Kumar G, Kumari S, Biswas S, Padmanabhan P. Synthesis of silver nanoparticles by Bacillus clausii and computational profiling of nitrate reductase enzyme involved in production. Journal of Genetic Engineering and Biotechnology. 2018;16(2):527-36.

10. Pourali P, Yahyaei B. Wound healing property of a gel prepared by the combination of Pseudomonas aeruginosa alginate and Alhagi maurorum aqueous extract in rats. Dermatologic therapy. 2019;32(1):e12779.

11. Kotcherlakota R, Das S, Patra CR. Therapeutic applications of green-synthesized silver nanoparticles. Green Synthesis, Characterization and Applications of Nanoparticles: Elsevier; 2019. p. 389-428.

12. Maillard J-Y, Hartemann P. Silver as an antimicrobial: facts and gaps in knowledge. Critical reviews in microbiology. 2013;39(4):373-83.

13. Nanda A, Saravanan M. Nanomed.: Nanotechnol. Biol Med. 2009;5:452-6.

14. ADEOYE AO. ANTIMALARIAL ACTIVITIES OF AQUEOUS AND METHANOLIC EXTRACTS AND FRACTIONS OF Adansonia digitata STEM BARK IN Plasmodium berghei-INFECTED MICE: University of Ilorin; 2017.

15. Kim JS, Kuk E, Yu KN, Kim J-H, Park SJ, Lee HJ, et al. Antimicrobial effects of silver nanoparticles. Nanomedicine: Nanotechnology, biology and medicine. 2007;3(1):95-101.