Chapter 8

Synthesis and Optimizatin of Bioactive Silver Nanoparticles Using *Staphylococcus Aureus* Strain

Şehmuz Asaf Başaran[1]

Nurcihan Hacıoğlu Doğru[2]

Abstract

The term nanoparticle refers to particles with dimensions of 100 nm and below. Unlike large structured substances, nanoparticles have their own physical, chemical, electronic, mechanical, magnetic, thermal, dielectric, optical and biological properties with their nano-sized structure. Due to these properties of nanoparticles, synthesizing nanoparticles has gained a very important dimension in recent years. Bacteria are more preferred for the synthesis of nanoparticles due to their abundant diversity in nature, ease of application, high activity, rapid growth rates, easy reproduction, pH and pressure.

In this study, it was aimed to use Staphylococcus aureus isolate, isolated from the soils in Ezine district in silver nanoparticle synthesis, to optimize the synthesis steps and to reveal the antimicrobial activity of the synthesized nanoparticle. Uv-Vis and Scanning Electron Microscope (SEM) analyses and the color change in the reaction proved that the isolate has the property of nanoparticle synthesis. However, the effect of AgNO3 concentration, pH and temperature on the synthesis steps was investigated. According to the results obtained, the isolate reaches the optimum production process at a concentration of 1 mM AgNO3, at 37 oC, pH 10. In addition, the obtained particle showed higher antagonistic effect than the comparative antibiotic against the bacteria Pseudomonas aeruginosa ATCC 27853, Staphylococcus haemolyticus and Acinetobacter baumannii ATCC 19606.

1 Undergraduate student., Çanakkale Onsekiz Mart University, Faculty of Sciences, Department of Biology, Çanakkale, Türkiye, ORCID: 0000-0002-1417-0444, asaf_248@hotmail.com

2 Prof. Dr., Çanakkale Onsekiz Mart University, Faculty of Sciences, Department of Biology, Çanakkale, Türkiye, ORCID: 0000-0002-5812-9398, nurcihan.n@gmail.com

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Introduction

The term nanoparticle refers to particles with dimensions of 100 nm or less. Unlike large-structured materials, nanoparticles have unique physical, chemical, electronic, mechanical, magnetic, thermal, dielectric, optical and biological properties with their nano-sized structures (Portakal, 2008; Durán et al., 2011). Thanks to their unique structure, metal nanoparticles have a high function for use in the electronics and materials industry and allow the development of new methods in medical applications such as the production of antimicrobial substances, the delivery of drugs, the diagnosis and treatment of diseases (Gaikwad et al., 2013; Park et al., 2016; Singh et al., 2016; Składanowski et al., 2017).

Due to these properties of nanoparticles, synthesizing them has gained a very important dimension in recent years. Physical and chemical methods are applied in the production of nanoparticles. Hydrothermal/solvothermal method, sol-gel method, ultraviolet irradiation technique, aerosol technologies, lithography, laser ablation, ultrasonic applications, photochemical reduction techniques, stencil method, microwave approach and chemical vapor deposition are some of these methods. Chemical methods are expensive and unstable methods that require high energy, reducing/stabilizing agents and toxic chemicals. In addition, the produced nanoparticles may have biological side effects (Jena et al., 2015; Bakhshi and Hosseini, 2016; Park et al., 2016; Składanowski et al., 2017). Therefore, non-biological toxic, environmentally friendly and economical syntheses come to the fore in nanoparticle production. Plants, fungi, actinomycetes and bacteria can be used for nanoparticle synthesis. These can produce non-toxic metal nanoparticles such as gold, silver, platinum, iron sulfide, cadmium sulfide, selenium, zinc oxide; copper (Zhang et al., 2016). Thanks to their detoxification mechanism, microorganisms bind metals with proteins and peptides in their structures and eliminate the toxic effect of metals. Bacteria are more preferred for the synthesis of nanoparticles due to their abundant diversity in nature, ease of application, high efficiency, rapid growth rates, easy reproduction, pH and pressure.

It has been reported that bacteria belonging to *Pseudomonas*, *Klebsiella*, *Escherichia*, *Lactobacillus*, *Corynebacterium*, *Magnetospirillum*, *Clostridium* genera, especially *Bacillus*, are capable of synthesizing various nanoparticles. Among the nanoparticles synthesized intracellularly by bacteria are gold, silver, selenium, palladium, iron oxide, magnetite, cadmium sulfide, zinc sulfide and zinc oxide (Sweeney et al., 2004; Narayanan and Sakthivel, 2010; Harikrishnan et al., 2014; Lampis et al., 2014; Markus et al., 2016).

Metal nanoparticles synthesized by bacteria have the opportunity to be used in many industrial areas, especially medicine, pharmacy and food, with their non-toxic structure and environmental friendliness (Wei et al., 2012).

Silver nanoparticles (AgNP) were known to have an antimicrobial effect on bacteria such as *Staphylococcus aureus*, *Vibrio parahaemolyticus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, *Bacillus cereus*, *Serratia entomophila*, *Klebsiella planticola*, *Klebsiella pneumoniae* and *Streptococcus* sp. (Du and Yi, 2016; Park et al., 2016; Rajeshkumar et al., 2016). It has been reported that silver nanoparticles, which also show antifungal properties, have inhibitory properties against *Aspergillus fumigatus*, *Aspergillus niger*, *Candida albicans* and *Candida tropicalis* (Abouelkheir et al., 2016; Du and Yi, 2016). In studies with bacteriologically produced some silver nanoparticles, has been observed that these particles have antioxidant and larvicidal activities such as catching DDPH radical and reducing iron ions (Lateef et al., 2015).

The primary objective of this research is to determine and optimize the nanoparticle synthesis by *Staphylococcus aureus* isolate obtained from Ezine soils. In addition, the antimicrobial activity of the synthesized AgNP was determined.

Material and Methods

Synthesis of AgNP by S. aureus

The bacterial isolates were isolated from Ezine districts soil. A series of basic biochemical tests were performed to identification of isolates according to Tamer et al. (1989). Bacterial strains were maintained on Luria–Bertani agar (LB agar) Petri dishes at 22 °C. Analytical grade $AgNO_3$ was purchased from Sigma Aldrich (Milan, Italy).

Single colonies of *S. aureus* strain from overnight LB agar Petri dishes served as the inocula for 100 mL LB broth cultures in 500 mL Erlenmeyer flasks. The cultures were incubated at 37 °C in a shaker (200 rpm) for 24 h. AgNP biosynthesis was carried out by resuspending 200 mg of bacterial strain in 100 mL of deionized water. 1 mM of $AgNO_3$ was added to culture and the reaction mixture was incubated for 24 h in a rotator shaker at 150 rpm and 37 °C. Characterizations of AgNPs were done by Scanning Electron Microscopy (SEM) and Uv-Vis spectra (200-800 nm). SEM analysis was carried out as service procurement at Kastamonu University Central Laboratory (MERLAB).

Optimization of Synthesized AgNP

Determination of the effect of AgNO3 concentration on the biosynthesis of NPs

In order to determine the ideal concentration value for the production of Ag nanoparticles, 50 mL of *S. aureus* supernatant was removed from a pre-prepared solution of AgNO3 of 0.25, 0.5 and 1 mM to the incubator with agitation of 37 °C, 150 rpm. After 144 hours of incubation at 37°C in the agitated incubator (120 rpm), the samples were diluted by 1/5 with distilled water and absorbance measurement was made at a wavelength of 341 nm in the Uv-Vis spectrophotometer.

Determination of the effect of pH on the biosynthesis of NPs

From the prepared 50 mM solution of AgNO3, 11.53 mM was added to the supernatant, and with 2 N HCl or 2 N NaOH, the pH values were adjusted to 6, 7, 8, 9 and 10 respectively. Incubation was continued in the agitated incubator at 37 °C, at 120 rpm, for 144 hours. At the end of incubation, the samples taken were diluted with distilled water at a rate of 1/5 and absorbance measurement was made at a wavelength of 341 nm in the Uv-Vis spectrophotometer. The pH value of the sample in which the absorbance value was read the highest was determined as the optimum pH.

Determination of the effect of temperature on the biosynthesis of NPs

The sample prepared at optimum concentration and pH value was incubated in the shaken incubator (120 rpm) at four different temperatures: 30 °C, 33 °C, 37 °C and 40 °C. The samples taken were diluted by 1/5 using distilled water and their absorbance was measured at a wavelength of 341 nm in the Uv-Vis spectrophotometer. The temperature value of the sample with the highest absorbance value was determined as the optimum temperature.

Determination of the antimicrobial effect of AgNPs

Disc diffusion method was used to determine the antimicrobial effect of AgNP on bacteria (Subhapriya and Gomathipriya, 2018). A total of 8 microorganisms obtained from Çanakkale Onsekiz Mart University, Faculty of Science, Department of Biology, Basic and Industrial Microbiology Culture Collection were used in antimicrobial activity studies.

Results and Discussion

Reduction of Ag+ into AgNPs during exposure to *S. aureus* bacteria could be seen by the color change. The color of fresh culture of bacteria was yellow. However, after addition of $AgNO_3$ and incubation, the emulsion turned dark brown (Figure 1). The color changes in aqueous solutions are due to the surface plasmon resonance phenomenon (Shakibaie et al., 2015). The result obtained is evidence that *S. aureus* is a good reducing agent for AgNPs.

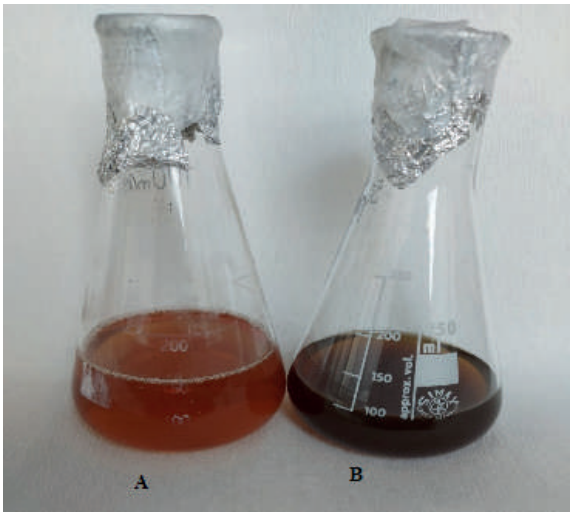

Figure 1. Colour changes of synthesized AgNP (A: Culture +*S. aureus****; B: Synthesized AgNP)***

The morphology of AgNPs was determined by SEM characterization analyses. SEM is an important technique used to investigate the surface morphologies of nanostructures. SEM images of the AgNP sample are given in Figure 2. Ag nanoparticles appear to be haphazardly dispersed on the platform in bacterial culture medium and have sizes ranging in the range of 49-89 nm. The fact that AgNP, which normally have spherical structure, have an enlarged (extended) structure that does not have a complete spherical structure in the study is thought to be due to the aggregation of two or more Ag nanoparticles during synthesis. This is in line with other studies in the literature (Tamuly et al., 2013).

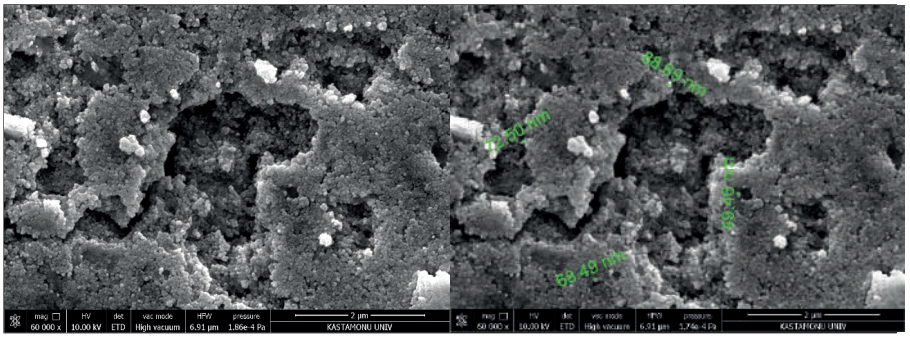

Figure 2. SEM images of synthesized AgNP

In the biosynthesis of AgNP, the most efficient production conditions were determined by optimizing the ambient pH, the concentration of the Ag solution and the temperature parameters. Three parallel studies were carried out in all of the applications. When the pH value was examined after incubation with 50 mL *S. aureus* from 0.25, 0.5 and 1 mM $AgNO_3$ solution, the pH value was 8. AgNP formation in the range of 396-387 nm was detected in the Uv-Vis spectrum (Figure 3). The maximum Uv-Vis absorbance value was observed by the culture supernatant at a concentration density of 1mM. Therefore, 1mM concentration was chosen as the optimum concentration.

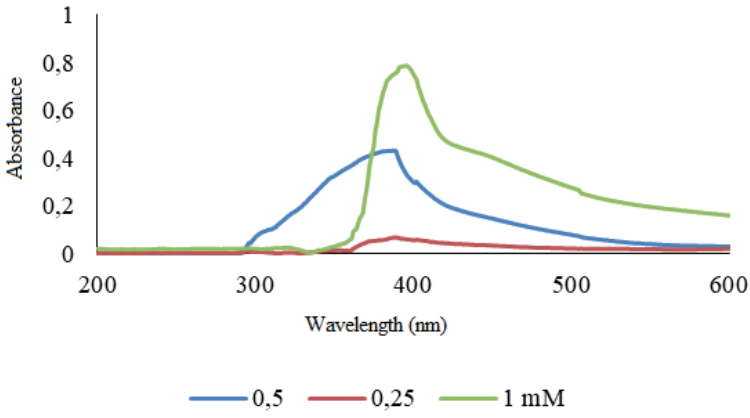

Figure 3. Uv-Vis spectra of different concentrations of $AgNO_3$ in AgNP synthesis

To study the effect of different pH on AgNP production; *S. aureus* cultures were treated with $AgNO_3$ prepared at optimum concentration. The pH of the samples was set to 5, 6, 7, 8, 9 and 10 respectively with 2 N HCl or 2 N NaOH. The samples treated with silver nitrate and adjusted to pH were removed to the incubator with 37 °C, 150 rpm shake. During the time with the addition of silver nitrate to cultures of different pH, a yellowish-brown color was observed in solution due to the surface plasmon resonance stimulation of AgNPs. It was observed that as the incubation period increased, the observed color became darker. AgNP formation in the range of 405-396 nm has been detected in the UV-VIS spectrum. It was observed that the absorbance value increased in direct proportion to the pH and the maximum Uv-Vis absorbance value was shown by the culture supernatant at pH 10 (Figure 4).

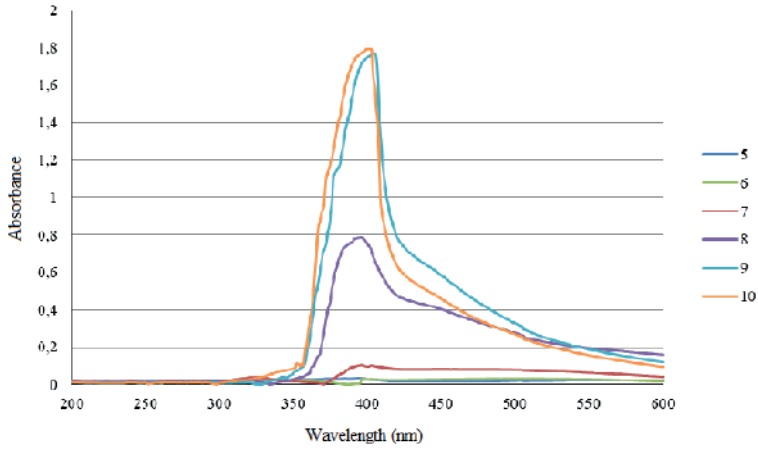

Figure 4. Spectrum graph of AgNP at different pH values

To study the effect of different temperature on AgNP production the samples were incubated at 3 different temperatures, 35, 37 and 40 °C. In the Uv-Vis spectrum, AgNP formation was detected at 396 nm. The maximum Uv-Vis absorbance value was observed to be demonstrated by the culture supernatant at 37 °C (Figure 5).

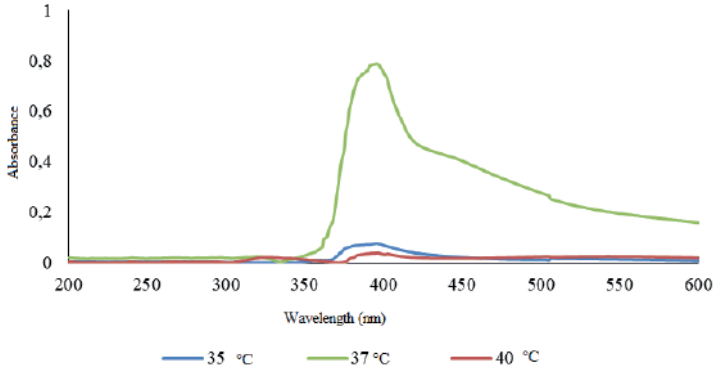

Figure 5. Uv-Vis spectra of AgNP at different temperatures

The antimicrobial activity results of AgNPs were given in Table 1. The zone diameters obtained against the bacteria *P. aeruginosa* ATCC 27853, *S. haemolyticus* and *A. baumannii* ATCC 19606 were higher than the comparison antibiotic P10 against the test cultures. This situation reveals the potential of the synthesized nanoparticle to become a new source of drugs.

Table 1. Antimicrobial activity results of AgNP

Test Cultures	*Disc Diffusion(mm)[a]	Control Antibiotics	
	AgNP	P10	NY100
E. coli NRRLB 3704	0	16.0	D
P. aeruginosa ATCC 27853	13	8.0	D
P. vulgaris ATCC 13315	11	13.0	D
B. subtilis ATCC 6633	0	14.0	D
S. haemolyticus ATCC 43252	19	14.0	D
A. baumannii ATCC 19606	15	12.0	D
S. aureus ATCC 6538P	0	15.0	D
C. albicans ATCC 10231	0	D	16.0

There has been no information in the literature on the synthesis of silver nanoparticle from *S. aureus* bacteria and the antimicrobial activity of the synthesized product. In one study, SeNPs (10-50 nm) produced by *Bacillus licheniformis* were found to have antimicrobial effects on some foodborne pathogenic bacteria (*B. cereus*, *E. faecalis*, *S. aureus*, *E. coli* O157:H7, *S. Typhimurium* and *S. Enteritidis*) (Khiralla and El-Deeb, 2015). In addition, biogenic SeNPs have been reported to have an antibiotic effect on *P. mirabilis*, *S. aureus* and *P. aeruginosa* (Shakibaie et al., 2015). According to the all this information, it can be said that it has the potential to be used as a new antimicrobial agent for antimicrobial agents coated with AgNPs.

Conclusion

The studies aimed to be carried out in the future are to carry out detailed studies for the comprehensive use of nanoparticle products synthesized by bacterial and all optimization conditions are revealed in medical, medical and industrial fields. The information obtained as a result of our pre-screening experiments suggests that AgNPs of *S. aureus* origin are of a quality to be used in more detailed research.

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