ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES SYNTHESIZED FROM BIOSURFACTANT

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ABSTRACT

The use of microbial surfactant for the synthesis of silver nanoparticles was suggested in the present work using biochemical methods. Using *Bacillus licheniformis*, which was isolated from the coastal region of Chennai, Tamilnadu, India, surfactant was synthesized. Surface tension measurements, the parafilm M test, and the drop-Collapse technique were used to screen the synthesis of biosurfactants. The inclusion of two reverse micelles that had already been produced in the presence of $NaBH_4$ as a reducing agent was used to create silver nanoparticles in situ in the water-in-oil microemulsion phase. In order to analyze the silver nanoparticles synthesis from surfactant, UV-visible spectroscopy, scanning electron microscopy and transmission electron microscopy were used. A surface plasmon resonance vibration band was visible at 432 nm in the UV-vis spectra. Silver nanoparticles synthesized from biosurfactant from *Bacillus licheniformis* ranged in size from 28 to 44 nm, according to SEM images. The silver nanoparticles on the films ranged in size from 15 to 32 nm, according to TEM images, which showed a significant distribution of smaller particles. *Salmonella typhi* (301.15 mm) and *Staphylococcus aureus* (201.15 mm) had the highest zone of inhibition values for silver nanoparticles antibacterial activity.

Key words: Biosurfactant, silver nanoparticles, Bacillus licheniformis, UV- Visible spectroscopy, Scanning electron microscopy, Transmission electron microscopy

INTRODUCTION

Nanoparticles exhibit well-defined chemical, optical, and mechanical properties, so the use of them is expanding in the twenty-first century. Due to increasing microbial resistance to metal ions, antibiotics, and the emergence of resistant strains (Anandaraj and Thivakaran, 2010; Arima *et al.,* 1968), metallic nanoparticles are the most promising because they exhibit good antibacterial properties due to their large surface area to volume ratio. There are various kinds of nanoparticles, and metal nanoparticles in particular have a significant impact. Due to their distinct physical, chemical, and biological characteristics in comparison to their macro-scaled counterparts, silver nanoparticles (Ag-NPs or nano silver) are attracting increasing attention among them (Baker *at al.,* 2005; Banat *et al.,* 2010).

To create silver nanoparticles, a variety of methods have been used, including chemical reduction, photochemical reduction, reverse micelle based and lamellar liquid crystals approaches, aerosol techniques, and an electrostatic spraying method. Considering that the reverse micelles method was used to create metal nanoparticles (Carrillo *et al.,* 1996). Inert gas condensation and co-condensation strategies for the synthesis of nanoparticles have been reported (Shahvardi *et al.,* 2007). At modest concentrations, the antibacterial activity of the nanoparticles was found. TEM and high angled annular dark field microscopy were used to study the impact of silver nanoparticles on Gram-negative bacteria with a size range of 1 to 100 nm (Chopra, 2007).

The biosurfactant is a key component in the creation of many nanoparticles in the field of nanotechnology, in addition to its use in the environment (Desai and Banat, 1997). As a promising technique for stabilizing nanoparticles, surfactant-mediated nanoparticle production is becoming more popular. A low-cost biosurfactant can be employed for nanoparticle synthesis as a non-toxic, biodegradable stabilizing agent, as demonstrated by simpler method for producing nanoparticles when compared to systems that currently require whole organisms or partially purified biological extracts (Duran *et al.,* 2007; Farias *et al.,* 2014). The feasibility of creating silver nanoparticles in a water-in-oil microemulsion that is stabilized by a low-cost biosurfactant is studied in the present research. The generated silver nanoparticles are identified using a Transmission Electron Microscope (TEM) and UV-vis absorption spectrum (Horowitz *et al.,* 1990).

At low concentrations, the nanoparticles were observed to have antimicrobial action. The surface area to volume ratio of silver nanoparticles was thought to be a factor in the antibacterial activity of these particles. Smaller particles had greater surface area to volume ratios and, as a result, more effective antibacterial activity (Kluge *et al.,*

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1989; McInerney *et al.,* 1990). Silver nanoparticles' antimicrobial activity against *E. coli,* a representative example of gram-negative bacteria, has been documented. Additionally, it was shown that the silver nanoparticles interact with the components of bacterial membranes and harm the cell. A RNA virus was researched for how silver ions and UV radiation interact to effectively increase the efficiency of UV radiation Rai *et al.,* 2009).

METHODOLOGY

Screening of biosurfactant (Horowitz *et al.,* 1990)

Different marine bacterial strains (1x106CFU/ml) were inoculated in 5 mL of nutrient broth for biosurfactant screening, and the mixture was then incubated at 37°C in a rotary shaker incubator at 145 rpm for 24 hours. Each culture was incubated under the aforementioned conditions for 72 hours before being transferred to 100 cc of Zobell marine media containing 2% crude oil as a carbon source. In order to identify strains that produce biosurfactants effectively, the drop collapse test, the parafilm M test, and surface tension measurements were used in the screening process. By using morphological, biochemical, and molecular based techniques, screened biosurfactant was found and used for nanoparticle manufacturing.

Production of silver nanoparticles from biosurfactant (Rajendran *et al.,* 2011)

The inclusion of two reverse micelles that had already been produced in the presence of NaBH4 as a reducing agent was used to create silver nanoparticles in situ in the water-in-oil microemulsion phase. Up to 0.5 mL of 0.05 Mol/L aqueous $AgNO_3$ solution, 3.0 g of *Bacillus licehniformi*s-produced biosurfactant, 1.5 g of n-butanol, and 0.5 g of n-heptane must all be combined and rapidly agitated at room temperature until uniform reverse micelles are formed. Then, in place of aqueous $AgNO_3$ solution, reverse micelles were also created using 0.5 ml of 0.1 mol/l aqueous $NaBH_4$ solution. The two different reverse micelles were then combined and agitated at 10,000 g for 60 minute. Additionally, ethanol (0.5 ml ethanol for 1 ml reverse micelles) was used to break up the reverse micelles. A particle that breaks down tends to precipitate from the solution. By centrifugation at 15,000 X g, the silver particles that had precipitated were extracted. Silver nanoparticles of various sizes are produced as a result of this process. After being sonicated in 10 ml of n-heptane solution, the particles were kept for later research.

UV-Visible Spectroscopy *(*Sharma *et al.,* 2009**)**

The UV Visible absorption spectrometer (ELICO SL 244) was used to detect absorption spectra at room temperature in the wavelength range of 200 to 800 nm in the dispersion mode in order to characterize the optical properties of the produced silver nanoparticles.

Scanning Electron Microscopy (Siegel, 1993**)**

Using a sputter coater, a conductive metal, such as gold, should be coated on the nanoparticle solution before placing it on a sample holder for scanning electron microscopy analysis. Following that, a finely focused electron beam was utilized to scan the material. The secondary electrons emitted from the sample surface were used to determine its surface properties. The polymer can be harmed by the electron beam, and the nanoparticles require to be able to withstand vacuum. The results from dynamic light scattering were equivalent to the mean size found by SEM. Using a scanning electron microscope, the morphological characteristics of the nanoparticle, including particle size, shape, and topography, were examined.

Transmission Electron Microscopy (TEM) (Besson and Michel, 1992)

A beam of electrons is focused onto a specimen during transmission electron microscopy (TEM), which produces an enlarged image that can be seen on a fluorescent screen, a layer of photographic film, or recorded by a CCD camera. Imaging mode used to study the microstructure. The produced silver nanoparticles used in our investigation were lyophilized and dissolved in 100% pure ethanol. After being dispersed in ethanol, the particles were deposited on a copper grid using sonication. That was examined using a 10,000X transmission electron microscope (JEM-2100F LaB6, USA).

Stability studies of synthesized silver nano particles (Portilla-Rivera *et al.,* 2008)

The absorption spectra of the solution on different days were studied in order to maintain the stability of silver nanoparticles particles. The produced silver nanoparticles Plasmon absorption bands have been studied after increasing the time by 1, 30, and 60 days. No passivator was added to the system at any point during the entire chemical process.

Antibacterial activity of silver nanoparticles from biosurfactant (Rai *et al.,* 2009)

Agar well diffusion method was used to assess the antibacterial activity of silver nanoparticles from biosurfactant against *Staphylococcus aureus* and *Salmonella typhi*. Each test strain was inoculated on nutrient broth (NB) medium, and they were incubated overnight at 37°C. From each culture, 500µl was inoculated in Muller Hinton agar plates by flooding surface and spreading uniformly on the plates. Under the sterile conditions of a laminar air flow, the surplus liquid broth was allowed to air dry. Each plate had two wells that were created with a sterile borer. The wells were inoculated with 20 µL of nanoparticles and ampicillin and incubated for 24-48 hours. The biosurfactant's antibacterial activity is indicated by the formation of clear zones.

RESULTS AND DISCUSSION

Figure 1 depicts the optical absorption spectra of the silver nanoparticles produced by *Bacillus licheniformis* biosurfactant. As seen in the image, the homogeneous size distribution of the sample led to the narrow band edge (432 nm) in the optical absorption of the silver nanoparticles. The final product's nano-sized creation, which was brought on by the quantum confinement effect, was confirmed when the absorption edge was relocated to the lower wavelength area. Effective mass approximation calculations revealed that the material's optical band gap was 3.7 eV (432 nm), which was greater than the band gap of bulk silver nitrate (3.1 eV). Light with a wavelength below this provides sufficient energy to excite electrons, which allows silver nitrate to absorb it. On the other hand, longer wavelength light that was greater in energy (towards the visible light) than the band gap will not be absorbed. The size of the nanoparticles was validated by the optical absorption spectra of silver nanoparticles created using biosurfactant from *Bacillus licheniformis (*Horowitz *et al.,* 1990).

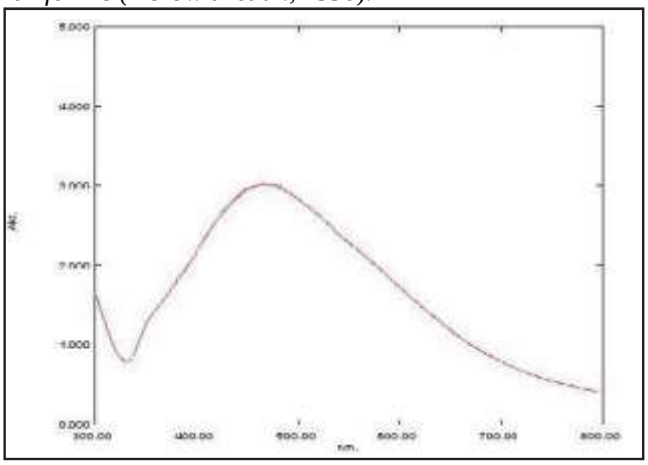


Fig. 1. UV-Visible Absorption spectrum of silver nanoparticles synthesizedusing biosurfactant from *Bacillus licheniformis.*

Scanning Electron Microscope

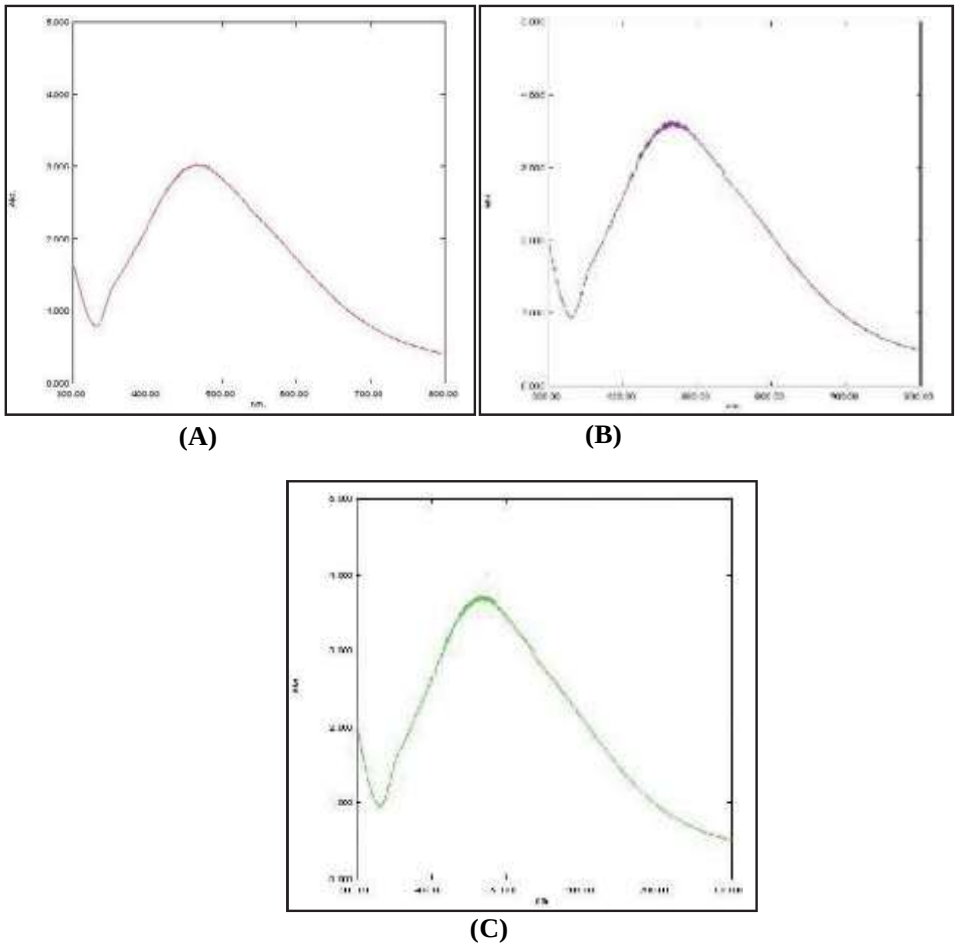
Scanning electron microscope was used to investigate the surface morphological characteristics of the silver nanoparticles, including particle size, shape, and topography. It demonstrated that the particles were oval, distinct and regular. The particles surfaces didn't smooth. The biosurfactant from *Bacillus licheniformis* was used to synthesize silver nanoparticles with a size range of 28 to 44 nm. The particles had a smooth surface and were round. The colloidal suspension had an aggregate of particles that were spherical in form and had a rough surface. The SEM image showed that the particles were synthesized and stabilized by the biosurfactant.

High Resolution Transmission Electron Micrograph of Silver nanoparticles

The silver Nanoparticles undergo HR-TEM examination to determine their unique size and form. A HR-TEM images showed that the synthesis of silver nano particles. The silver nanoparticles from *Bacillus lichenformis* were scattered in huge numbers of smaller particles on the films, with sizes ranging from 15 to 32 nm. This shows that the biosurfactant-stabilized silver nanoparticle distribution was largely uniform. The continuous ring patterns that result from polycrystalline states or from multiple crystallites connected to the surface of single particles were revealed by the Selected Area Electron Diffraction (SAED) analysis. The materials in the samples of silver nanoparticles displayed a bright ring pattern that indicated a high density of crystallites.

Stability study of silver nanoparticles

After one day, the silver nanoparticles created with biosurfactant from *Bacillus licniformis* had a comparatively strong absorption peak at 432 nm. After a day, silver nanoparticles made with biosurfactant from *Bacillus licniformis* had a rather strong absorption peak at 406 nm (Fig. 2).



(A) **(B)**

(C)

Fig. 2. UV–Vis absorption spectra of silver nanoparticles synthesized frombiosurfactant from *Bacillus lichrniformis* in the n-heptane solution at room temperature for 1(A), 30 (B) and 60 (C) days.

The decrease in absorbance, which suggests a slight aggregation of silver nanoparticles, there are no obvious changes in the location and symmetry of the absorption peak. No passivator was added to the system at any point during the whole chemical process. It demonstrates that the silver nanoparticle solution made in such proportional reverse micelles can last for at least two months with remarkably little deterioration. The remaining rhamnolipid and lipopeptide in the solution were considered the stabilizers because they provide a steric barrier around the particles to avoid significant electrostatic aggregation. Reverse micelles system was created by in situ synthesis of silver nanoparticles in the water-in-oil microemulsion phase. Further characterization was carried out on the silver nanoparticles that were synthesized from biosurfactant. From UV- visible spectroscopy the optical absorption of the silver synthesized using from PBSC1 possess a narrow band edge (432 nm), which developed from the uniform sized particle distribution of the sample. Silver nanoparticles (SNPs) synthesized with biosurfactant from KBSB1 were found to have an optical absorption value of 405 nm, which was a direct result of the quantum confinement effect for small particle size (Rai *et al.,* 2009).

Antibacterial activity of silver nanoparticles from biosurfactant

In this method overnight nutrient broth cultures of each of the two strains were prepared and a lawn of culture was made on Muller Hinton agar plates. Silver nanoparticles and ampicillin dissolved in distilled water

were used as sample and positive control. After 24 hours of incubation, a clear zone of inhibition was observed against two test strains inoculated with silver nanoparticle and ampicillin. Clear zone was indicated the presence of antibacterial activity of nanoparticles from biosurfactant. The zone of inhibition values of silver nanoparticles were *Staphylococcus aureus* (20 ± 1.15mm) and *Salmonella typhi* (30 ± 1.15 mm). From the above results, it was observed that the silver nanoparticles from biosurfactant produced highest zone of inhibition against *Salmonella typhi*. Mechanistic study of inhibition of silver nanoparticles against two strains of bacteria, *S. aureus* and *E. coli* were analyzed. The silver nanoparticles enter into the bacterial cells by penetrating through the cell wall and consequently turn the DNA into condensed form which reacts with the thiol group proteins and result in cell death. The silver ions also interfere with the replication process (McInereny *et al.*, 1990).

CONCLUSION

Studies conducted to use biosurfactant screened from *Bacillus licheniformis* as a silver nanoparticle synthesizer and stabilizer. The studies had found promising results. The synthesized silver nanoparticles were further characterized. From UV- visible spectroscopy the optical absorption of the silver synthesized using from *Bacillus licheniformis* possess a narrow band edge (432 nm), which developed from the uniform sized particle distribution of the sample. The SEM and TEM images revealed that the particles were synthesized and stabilized by the biosurfactant. Antibacterial activity of silver nano particles showed that the zone of inhibition values of *Staphylococcus aureus* (20 ± 1.15mm) and *Salmonella typhi* (30 ± 1.15 mm). This study concluded that the biosurfactant produced by *Bacillus licheniformis* can be applied for medical field.

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CONFLICT OF INTEREST

No conflict of interest.

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