



Biosynthesis of Nanoparticles and their Antibacterial Properties

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ABSTRACT

Diatoms are a rich source of metabolites with numerous uses, and because of their silica frustules, diatoms' silver nanoparticle (AgNP) has tremendous therapeutic potential for use against pathogenic microorganisms. In the current investigation, AgNP was synthesised using *Chaetoceros* sp., *Skeletonema* sp., and *Thalassiosira* sp. According to DLS data, the average size of the synthesised AgNP particles was 149.03 3.0 nm, 186.73 4.9 nm, and 239.46 44.3 nm, but SEM data showed 148.3 46.8 nm, 238.0 60.9 nm, and 359.8 92.33 nm, respectively. AgNP exhibiting a prominent peak of Ag⁺ ion within the spectra is strongly supported by EDX analysis. High negative zeta potential values suggest significant stabilisation even three months later. *Aeromonas* sp., *Escherichia coli*, and other bacteria were used to assess the biosynthesised AgNP's antibacterial activity. Broad-spectrum antibacterial action is seen in *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Bacillus subtilis*. Due to its low toxicity and biodegradable nature, this work stimulates the synthesis of diatom-based AgNP for a number of applications.

Keywords: Antibacterial assay; Diatoms; Green synthesis; Nanotechnology; Biodegradable

INTRODUCTION

The outstanding connection between technology and medical sciences opens up new fronts in the nanotechnology industry, which is constantly expanding. By altering the atomic or molecular structures of bulk materials on a nanoscale, nano-biotechnology is an emerging field that deals with the production of nanoparticles containing biological components [1]. Due to their distinctive qualities, such as their high surface reaction activity, high surface area to volume ratio, and good thermal and mechanical stability, metal-based nanoparticles have garnered a lot of attention and appear to be the foundation of the future generation of technology. Although physio-chemical techniques such sol-gel, chemical, photochemical reduction, electrochemical approach, laser ablation, ion sputtering, etc. have been suggested for the production of AgNP [2]. However, these approaches have a number of shortcomings, including Due to the significant risk involved, sophisticated synthetic processes, expensive and poisonous ingredients, and the emission of hazardous byproducts. Contrarily, biological synthesis is highly sought after since it is non-toxic, environmentally benign, biocompatible, and economically viable [3]. Due to their superior reducing and capping activity, naturally occurring agents such as plant extracts, algae, fungi, bacteria, yeast, enzymes, biopolymers, etc. are now frequently utilised in the creation of nanoparticles. The emerging and intriguing field of "phyconanotechnology" offers numerous opportunities for the production of nanoparticles made from algae. The most reliable mechanism for the environmentally

friendly synthesis of nanoparticles, whether they are metallic nanoparticles, nanostructured polymers, or a wide variety of novel nanomaterials, is diatom algae [4].

Diatoms are eukaryotic, unicellular microalgae that are typically found in water and belong to the class Bacillariophyceae with a size range of 2200 nm. The number of diatom species discovered worldwide exceeds 200,000. They act as a food web for the entire aquatic ecosystem and are crucial to overall carbon fixation. The silica cell wall of diatoms, known as frustules, is their most distinctive trait. They absorb silica from the environment at a concentration of less than 1 mM, and silicon acid transporters then condense it into their cell wall (SITs). It has been claimed that diatoms contain antibacterial, anticancer, antioxidant, and anti-inflammatory compounds, among other things [5]. Diatoms are an exceptional aquatic organism for nanotechnology research and applications as a result of all these characteristics. Since ancient times, silver (Ag) metal has been used for a variety of purposes, including as a wound-healing agent in the form of Ag plates, a treatment for ulcers, and a neonatal eye drop containing 1% silver nitrate (AgNO₃) to treat eye infections.

MATERIALS AND METHODS

Despite being the best conductor of electricity, its usage in the electrical industry is limited by its expensive cost. The AgNP performs a significant and highly effective function among gold (Au), silver (Ag), palladium (Pd), platinum (Pt), iron (Fe),

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cadmium (Cd), titanium oxide (TiO₂), zinc oxide (ZnO), and Ag-Au bimetallic nanoparticles because it has distinct thermal, optical, electrical, and biocidal capabilities [6]. It is crucial to find a new method to manage dangerous bacteria because they are now utilised in the medical field to destroy disease-causing microbes that have developed resistance to various antibiotics. According to reports, AgNP interacts with bacteria's cell wall and destroys it, which prevents protein production and slows down bacterial development. Additionally, AgNP is used in the biomedical industry, medicines, cosmetics, drinks, electronics components, dentistry, and wound healing.

Preparation of Diatom Culture

Three diatoms from the group Bacillariophyceae, *Chaetoceros* sp., *Skeletonema* sp., and *Thalassiosira* sp., were isolated for this study from the coasts of Vishakhapatnam and Rameshwaram, India (928760 N, 7931290 E). The species were kept in the Diatom Research Laboratory, AUUP, Noida, India, in 500 mL Erlenmeyer flasks containing synthetic seawater enriched with f/2-Si media, at pH 8.3, and maintained in 12:12 light and dark conditions at a temperature of 23°C with a luminous intensity of 100 [7]. Due to its strong antibacterial activity, biologically produced AgNP has been in high demand as nano-silver has become the focal focus of the nano-industry. Cyanophyceae, Chlorophyceae, Rhodophyceae, and Phaeophyceae are some of the algae that can produce AgNP, but diatoms (Bacillariophyceae) are the source of the least amount of research on AgNP since they increase the ability of Ag to kill dangerous microorganisms. In earlier research, *Navicula* was used to synthesise AgNP for ammonia sensing. In an effort to promote the use of AgNP for research and development, synthesised AgNP from *Phaeodactylum tricornutum*. Others have researched the harmful effects of AgNP in the diatom *Thalassiosira pseudonana* and *Cyanobacterium*. Garcia et al. explored the detection of AgNPs in *Thalassiosira pseudonana*.

The *Chaetoceros curvisetus*, *Trichodesmium erythraeum*, which produces blooms, was used to make AgNP. investigated its biological potential. For various biotechnological uses, AgNP were also produced from *Amphora*, *Navicula atamus*, and *Diadismis gallica* produced peptide-mediated AgNP on the surface of the diatom. All of these have demonstrated that biological synthesis [8].

Synthesis of AgNP from diatoms

Using AgNO₃ as a precursor, the AgNP was created from three different species of diatoms (*Chaetoceros* sp., *Skeletonema* sp., and *Thalassiosira* sp.). Each diatom species' collected biomass was gradually added to the aqueous solution containing AgNO₃ while being stirred continuously. Initial colour density varies from colourless to pale pink in 12 hours, then gradually shifts to red brown after 24 to 48 hours of incubation at room temperature with light. The color's changing intensity over time proves AgNP [9].

Characterization of Silver Nanoparticles

By measuring the absorption spectra from 300 to 800 nm with a quartz cuvette with a 1 cm path length and an AgNO₃ control, the biosynthesis of AgNP was verified. To detect the bio reduction of Ag⁺ ions in the aqueous solution, a known volume of the reddish-brown reaction mixture was obtained, and its spectra were captured. The surface plasmon resonance (SPR) band that is characteristic of the biosynthesized AgNPs was seen, confirming their synthesis. On a Malvern Zetasizer - Nano ZS (Malvern, UK) equipment with

backscatter detection (173), run by Dispersion Technology Software, the particle size distribution and zeta potential were computed using Debye-formula Scherrer's ($L = 0.9 \lambda / b \cosh h$) (DTS 5.03, Malvern, UK). To avoid air bubbles, a 1 mL aliquot of the reaction mixtures was carefully placed in a standard cell with electrodes on both sides of the cell holder providing the necessary voltage for the electrophoresis procedure. At 25 C, the instrument automatically calculated the zeta potential using the Henry equation.

Antibacterial assay

Both the agar well diffusion method and the microdilution assay method were used to test AgNP's antibacterial activity against Gram-positive (*Bacillus subtilis*, *Streptococcus pneumoniae*, and *Staphylococcus aureus*) and Gram-negative (*Aeromonas* and *E. coli*) bacteria that were obtained from the IMTECH culture collection centre in Chandigarh, India. In a nutshell, bacteria were introduced to test tubes containing 10 mL of broth and allowed to incubate overnight at 32 C in a bacteriology incubator (Relitech, India). The bacteria were further diluted to match 0.5 Mc Farland standard turbidity after being cultivated overnight. According to the Clinical Laboratory Standard Institute, a microdilution assay was carried out, and broth was used to generate the serial dilution. Similar to this, the bacteria were disseminated on agar plates in the agar well diffusion method, and then wells were created using sterile tip of a micropipette. Approximately 50 cc of each AgNP, together with AgNO₃ as a control, were put into each well. The plates were then incubated at 37 C for the following day [10].

RESULT AND DISCUSSIONS

Here, AgNO₃ and the bacteria *Chaetoceros* sp., *Skeletonema* sp., and *Thalassiosira* sp. were used to create AgNP. The harvested biomass was gently incorporated into the AgNO₃ solution while being continuously stirred at room temperature. After 12 hours, the colour changed from being colourless to pale yellow, and after 48 hours of incubation, it progressively turned reddish-brown, as seen in Fig. 1. AgNO₃ solution used as a control showed no change in colour. A visible indicator of the beginning of the reaction, followed by the nucleation and development of nanoparticles in which nearby nucleonic particles join with one another to create thermodynamically stable AgNP, is the shift in hue. The biosynthesized AgNPs were submitted to UV-vis spectroscopy examination to further confirm the findings. Nanoparticle creation and stability. According to research reported in, the absorption spectra that exhibited a maximum absorption peak at λ_{max} 412 nm, 425 nm, and 430 nm, respectively, revealed biosynthesized AgNPs from *Chaetoceros* sp., *Skeletonema* sp., and *Thalassiosira* sp. Because the conduction band and valence band are close to one another in the case of metallic nanoparticles like AgNP, a broad peak is formed in the visual range as a result of the change in colour brought on by the excitation of free electrons that establish the SPR band. The bio reduction of Ag⁺ ions into stable Ago on diatoms cells occurs when light-sensitive biomolecules participate in the reaction pathway.

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