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Research Article

Biological Synthesis of Silver Nanoparticles by Cell-Free Extract of *Spirulina platensis*

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The present study explores biological synthesis of silver nanoparticles (AgNPs) using the cell-free extract of *Spirulina platensis*. Biosynthesised AgNPs were characterised by UV-Vis spectroscopy, SEM, TEM, and FTIR analysis and finally evaluated for antibacterial activity. Extracellular synthesis using aqueous extract of *S. platensis* showed the formation of well scattered, highly stable, spherical AgNPs with an average size of 30–50 nm. The size and morphology of the nanoparticles were confirmed by SEM and TEM analysis. FTIR and UV-Vis spectra showed that biomolecules, proteins and peptides, are mainly responsible for the formation and stabilisation of AgNPs. Furthermore, the synthesised nanoparticles exhibited high antibacterial activity against pathogenic Gram-negative, that is, *Escherichia coli*, MTCC-9721; *Proteus vulgaris*, MTCC-7299; *Klebsiella pneumoniae*, MTCC-9751, and Gram-positive, that is, *Staphylococcus aureus*, MTCC-9542; *S. epidermidis*, MTCC-2639; *Bacillus cereus*, MTCC-9017, bacteria. The AgNPs had shown maximum zone of inhibition (ZOI) that is 31.3 ± 1.11 in *P. vulgaris*. Use of such a microalgal system provides a simple, cost-effective alternative template for the biosynthesis of nanomaterials of silver in a large scale that could be of great use in biomedical applications.

1. Introduction

Particles with a size up to 100 nm are usually referred to as nanoparticles. Nanoparticles exhibit completely new or improved properties, based on specific characteristics, such as grain size, distribution, morphology, and higher surface to volume ratio if compared with larger particles of the bulk material [1]. A specific surface area is relevant to catalytic reactivity and other related properties, such as antimicrobial activity in silver nanoparticles (AgNPs).

Biological effectiveness of NPs enhances due to increase in specific surface area and surface energy [2]. Silver has long been documented as having an inhibitory effect on many bacterial strains and microorganisms commonly present in medical and industrial processes [3]. AgNPs proved to be effective as an antimicrobial agent even at a very low concentration and they inhibit the growth of antibiotic resistant bacteria. AgNPs interact with membrane proteins and DNA of bacteria, which have sulphur and phosphorous complex that have high affinity towards AgNPs [4]. The most widely used and known applications of silver and AgNPs prevent the infection of burns and open wounds in the medical industry and include Clinical Ultrasound Gel and topical ointments and creams [5]. Other widely used applications are medical devices and implants prepared with silver-impregnated polymers. In addition, silver containing consumer products, such as colloidal silver gel and silver embedded fabrics, are now used in sporting equipment [6]. AgNPs are used in low doses for antimicrobial treatment in comparison to standard antimicrobial agents [7].

Production of NPs may be achieved through different methods. Chemical methods are the most popular methods for the production of NPs. However, chemical methods cannot evade the use of toxic chemicals in the synthesis protocol. Since noble metal NPs, such as gold, silver, and platinum, are widely applied to human contact areas, there is a growing need to develop environmentally benign protocol of NPs synthesis that avoids the use of toxic chemicals. Biological methods of NPs synthesis using bacteria [2, 8], fungi [9,

10], plants [11–14], algae [15–17], sea weed [18], and lichen [19] have been recommended as ecofriendly alternatives to chemical and physical process.

Attachment of nanoparticles by cell wall of bacteria would be due to negative charges and specific functional groups on the bacterial surface. AgNPs after penetration into the bacterial cell may disturb the rigidity of cell wall or lipopolysaccharides membrane and inactivate enzymes functioning and their transport system and generation of H_2O_2 which results in bacterial death [20].

The synthesis of NPs of specific composition, self-assembly, and size is one of the most challenging areas of nanotechnology as it is strongly influenced by experimental conditions, the kinetics of interaction of metal ions with reducing agents, and adsorption processes of the stabilizing agent with metal NPs [10].

Among the microorganisms, microalgae have a tremendous role in bioremediation of toxic and precious metals and their biotransformation to different nontoxic forms [21]. They not only accumulate metals by chelation and chemical transformation, but are also reported to produce biomineral structures and metal NPs. However, most microorganisms that have been reported for synthesis of AgNPs are pathogenic to either plants or humans. So, over the years, researchers have turned to nonpathogenic microorganisms. Among the blue-green algae (cyanobacteria), *Anabaena*, *Calothrix*, *Leptolyngbya*, and *Nostoc ellipsosporum* have been reported to synthesise intracellular gold, silver, palladium, and platinum NPs [7]. The green microalga *C. vulgaris* has also been reported to produce gold, platinum, palladium, ruthenium, rhodium, and iridium nanoparticles, whereas the cell-free extract efficiently produces gold and silver nanoplates intracellularly [22]. Extracellular AgNP synthesis has been reported for the boiled extract of the brown seaweed *Padina tetrastromatica* [23].

S. platensis is a free floating filamentous cyanobacterium characterised by cylindrical, multicellular trichomes in an open, left-hand helix. It occurs naturally in tropical and subtropical lakes with high pH and high concentrations of carbonate and bicarbonate. In present study, we aim to test the ability of cell-free extracts of *S. platensis* to produce AgNPs in an aqueous system.

2. Materials and Methods

2.1. Microorganism and Culture Condition. The experimental organism *S. platensis* was isolated from Jal Mahal, Jaipur, Rajasthan (India), cultivated in Zarrouk's medium [24], under different temperature, and illuminated with white fluorescent lamps at a light intensity of 2,000 lux (Sharma et al. 2014). Conical flasks of 250 mL capacity were prepared containing 100 mL *S. platensis* culture and were shaken gently thrice a day to avoid clumping and enhance the growth.

2.2. Preparation of Microalgal Extract. Typically, 5 g (dry weight) *S. platensis* biomass was suspended in 100 mL of double distilled sterile water for 15 min at 100°C in an Erlenmeyer flask. After boiling, the mixture was cooled and centrifuged at 10,000 rpm for 15 min. Supernatant was collected and was stored at 4°C for further analysis.

2.3. Biosynthesis of Silver Nanoparticles. In the typically synthesis process of silver nanoparticles, add 2 mL of pure microalgal extract dropwise into the 100 mL of 1 mM of silver nitrate solution in 250 mL conical flask. The reaction mixture was kept at 60°C for 10 min under constant mechanical stirring. It was observed that reduction of Ag ions into AgNPs completed within 10 min, indicating rapid synthesis of AgNPs, and pH remains within 4.7–5.0 during the period of reaction. The colour change was noted and nanoparticles formation was monitored using UV-Vis spectrophotometer. The synthesised silver nanoparticles were centrifuged at 15,000 rpm for 20 min at 4°C, and discard the supernatant to collect the pellet. The pellet was washed with distilled water for several times to remove impurities and 90% ethanol to get pure AgNPs powder.

2.4. Characterisation of Prepared Nanoparticles. The characterisation of AgNPs was carried out by surface plasmon resonance band using a UV-Visible spectroscopy 1800 of Shimadzu, Kyoto, Japan. Micrograph of AgNPs was obtained by scanning electron microscope of SEM-EVO 18, Carl Zeiss 30 KeV SEM. TEM micrograph of the AgNPs was observed using the TEM instrument of TEM-Tecni G2-S twin FEI 200 KeV TEM. TEM device conducted at an increasing voltage of 200 kv. The FTIR spectrum was recorded on FTIR-Shimadzu IR Affinity 1. All measurements were carried out in the range of 400–4,000 cm^{-1} at a resolution of 4 cm^{-1} (Figure 2).

2.5. Antibacterial Activity of AgNPs. A turbid liquid sample of each bacterial strain with an OD of McFarland of 0.5 (1×10^8 CFU/mL) was prepared in an isotonic NaCl (0.85%) solution. Furthermore, this solution was diluted ten times (1×10^7 CFU/mL) and used as inoculums. 100 μL of bacterial suspension was prepared and inoculated on Mueller-Hinton Agar-II (MHA-II) plates by spread plate technique. The sterile disc of 6 mm in diameter (Hi-Media Laboratories Pvt. Ltd.) was impregnated with 50 μL of 1 mg/mL solution of nanoparticles in deionised water. The discs were evaporated at room temperature for 24 hours. Aqueous extract of deionised water was used as negative control and gentamicin (10 μg/disc) was used as positive control. The discs were gently pressed on MHA Petri Plates and incubated at 37°C for 24 hours. The zone of inhibition in the diameter of each disc was measured in millimetre (mm) using a Hi-Media Laboratories Pvt. Ltd. zone scale. The experiments were done in four replicates and mean values of ZOI were reported.

The AgNPs prepared by *S. platensis* were used to evaluate antibacterial activity against Gram (−) and Gram (+) bacteria (*Escherichia coli*, MTCC-9721; *Proteus vulgaris*, MTCC-7299; *Klebsiella pneumoniae*, MTCC-9751; *Staphylococcus aureus*, MTCC-9542; *S. epidermidis*, MTCC-2639; *Bacillus cereus*,

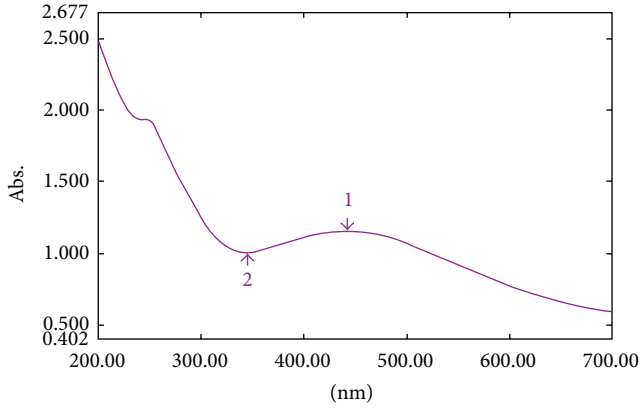


Figure 1: UV-Vis absorption spectra of AgNPs synthesised from *S. platensis*.

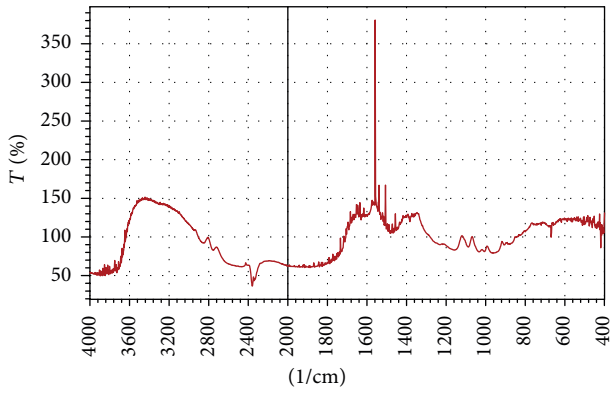


Figure 2: FTIR images of AgNPs generated using cell-free extracts of *S. platensis*.

MTCC-9017) on MHA-II plates by Kirby-Bauer disk diffusion method [25].

3. Results and Discussion

In this study, biological synthesis of AgNPs has been shown from cell-free aqueous extracts of *S. platensis*. These extracts when interacting with the silver nitrate salt solution form a dark brown solution due to the reduction of the silver ion to AgNPs followed by a colour change indicating the biotransformation of ionic silver to reduced silver and the subsequent formation of AgNPs in an aqueous medium. This reaction results in the biosynthesis of AgNPs showed by a colour change to dark brown from the light yellow seen at the beginning of the reaction (Figure 3). The colour change was monitored visually and the peak at 437 nm in the UV-visible spectra indicated the presence of AgNPs which may be due to the excitation of surface plasmon vibrations in AgNPs (Figure 1).

The morphological characteristics of biosynthesised AgNPs were studied by scanning electron microscope, using an instrument of SEM-EVO 18, Carl Zeiss 30 KeV SEM (Figure 4(a)). The TEM images showed that most of the particles are spherical in shape and do not create big agglomerates which indicated the monodispersed nature of NPs stabilised by a capping agent. The TEM images revealed that AgNPs are in the range of 30–50 nm (Figure 4(b)).

3.1. FTIR Spectrum Analysis. The FTIR spectrum of the AgNPs produced by cell-free extracts of *S. platensis* is shown in Figure 2. This spectrum shows the presence of band at 1533.89, 1558.48, 1639.49, 1652.99, and 3510.44 cm^{-1} corresponding to monosubstituted amide, nitro, primary amide, carboxylic, and alcohol group, respectively. The band at 1448.54 cm^{-1} is due to methylene scissoring vibrations present in the proteins. Largely, the observation confirms the presence of protein in AgNPs. FTIR spectroscopic study has confirmed that the monosubstituted amide of proteins has the stronger ability to bind metal, so that the proteins could most possibly form a coat covering the metal nanoparticles to prevent agglomeration of the particles and stabilizing in the medium. This data suggests that the biological molecules could probably perform the reduction and stabilisation of the AgNPs in the aqueous medium. These results confer the work of Awwad et al. [13].

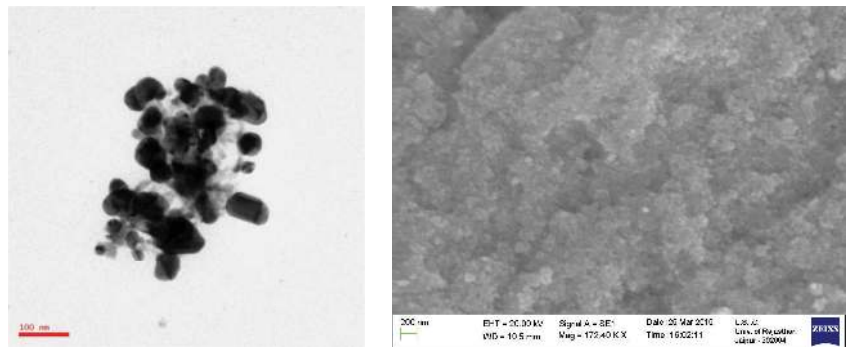
3.2. Antibacterial Activity of Ag Nanoparticles. Table 1 showed the four replicates experiments of zone of inhibition (mm) around the disc with cell-free aqueous extracts mediated synthesised silver AgNPs. The study revealed that AgNPs (50 μg/disk) had shown maximum inhibitory effect against *Proteus vulgaris*, MTCC-7299 and *Staphylococcus aureus*, MTCC-9542, that is, 31.3 ± 1.11 and 31.0 ± 0.71, followed by *Klebsiella pneumoniae*, MTCC-9751 (25.0 ± 0.91); *Escherichia coli*, MTCC-9721 (24.3 ± 0.48); *Bacillus cereus*, MTCC-9017 (24.3 ± 0.75); and *S. epidermidis*, MTCC-2639 (20.0 ± 0.41) as compared to gentamicin (+ control) (ZOI-22.0 mm), deionised water (– control) (ZOI- 0.0), *S. platensis* extracts (ZOI- 8.0 mm), and 1 mM silver nitrate solution (ZOI- 10.0 mm) against *Proteus vulgaris* (Figures 5(a)–5(f) and 6). The formations of free radicals from the surface of the silver nanoparticles were responsible for the antibacterial function. In addition, excess formation of reactive oxygen species (ROS) may lead to a breakdown of membrane function and increased permeability of the cell membrane or leakage of cell matters and morphological changes of bacterial cells and growth inhibition [26]. The charge of bacterial cell wall is negative because of dissociation of carboxylic groups on the cell surface [27]. Weak positive charges present on AgNPs are attracted towards negative charges [28]. In contrast, Sondi and Salopek-Sondi [29] suggested that the antibacterial effects of AgNPs on bacteria depended on the concentration of AgNPs and closely related with the development of "pits" on cell wall of bacteria. AgNPs interact with the thiol groups of bacterial proteins and may retard the replication of DNA [30]. It is consistent to state that binding of the nanoparticles to the bacteria depends on the surface area available for interaction. Nanoparticles have larger surface area available for interaction which enhances bactericidal effect than the large sized particles;

Table 1: Antibacterial activity of AgNPs.

Bacterial strain	AgNPs (1 mg/mL) (ZOI in mm)				Mean ± SE	Gentamicin (+) Control (ZOI in mm)	*S. platensis* extracts (ZOI in mm)	$AgNO_3$ (1 mM) (ZOI in mm)
Escherichia coli, MTCC-9721	24	23	25	25	24.3 ± 0.48	28	12	15
Proteus vulgaris, MTCC-7299	30	32	34	29	31.3 ± 1.11	22	8	10
Klebsiella pneumoniae, MTCC-9751	26	27	24	23	25.0 ± 0.91	16	8	11
Staphylococcus aureus, MTCC-9542	30	33	30	31	31.0 ± 0.71	25	9	12
S. epidermidis, MTCC-2639	21	20	19	20	20.0 ± 0.41	24	9	11
Bacillus cereus, MTCC-9017	23	25	26	23	24.3 ± 0.75	10	8	9

(a) (b) (c)

Figure 3: The pictures show the (a) *S. platensis* extracts, (b) $AgNO_3$ solution, and (c) AgNPs solution.



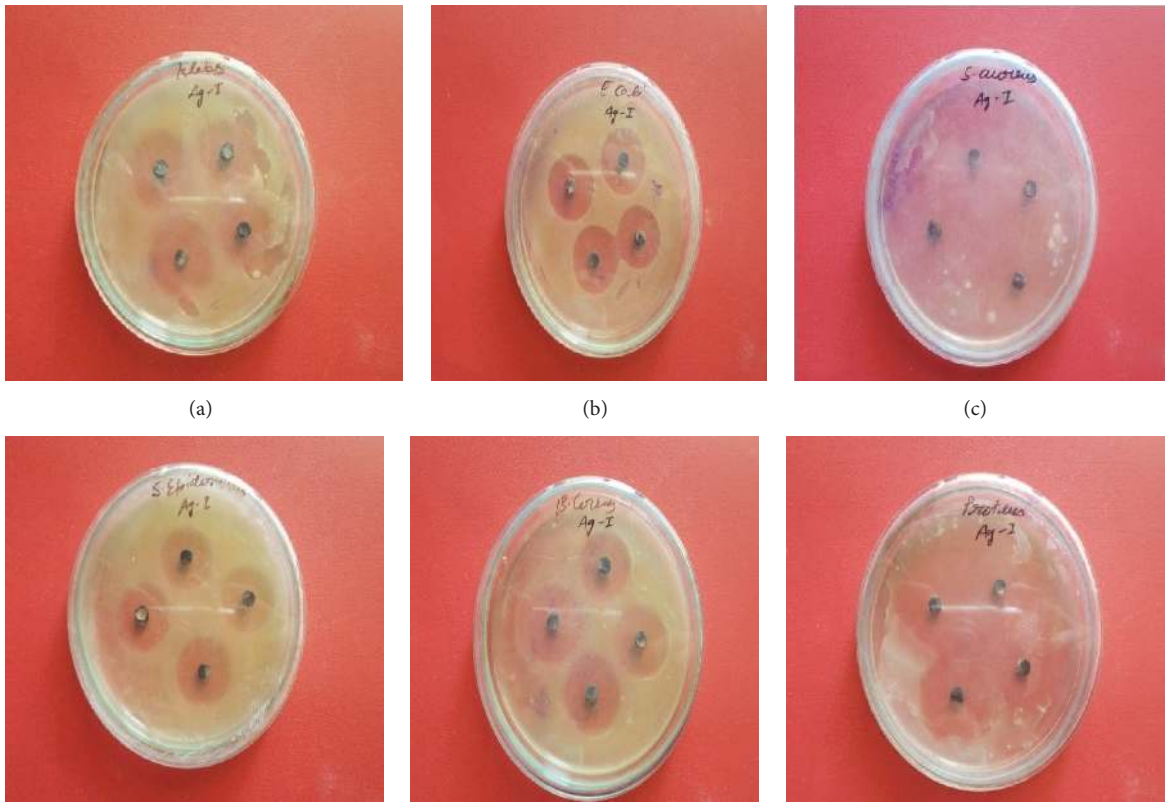
(a) (b)

Figure 4: (a) The SEM images and (b) TEM image of AgNPs synthesised by cell-free extracts of *S. platensis*.

hence AgNPs exhibit more toxicity to the microorganism [27].

4. Conclusion

It is concluded that the cell-free aqueous extracts produce stable AgNPs by reduction of aqueous Ag^+ ions in AgNPs. The utilisation of *S. platensis* biomass has various advantages like easy cultivation and availability. This biological method approach toward the synthesis of AgNPs has numerous benefits, that is, nontoxicity, cost effectiveness, rapid reduction, and economic viability. Future prospects of this research would be large scale production of AgNPs using *S. platensis* and ascertaining its efficacy against extensive spectrum of



(a) (b) (c)

(d) (e) (f)

Figure 5: (a–f) Antibacterial activities of AgNPs against (a) *Klebsiella pneumoniae*, (b) *Escherichia coli*, (c) *Staphylococcus aureus*, (d) *S. epidermidis*, (e) *Bacillus cereus*, and (f) *Proteus vulgaris*.

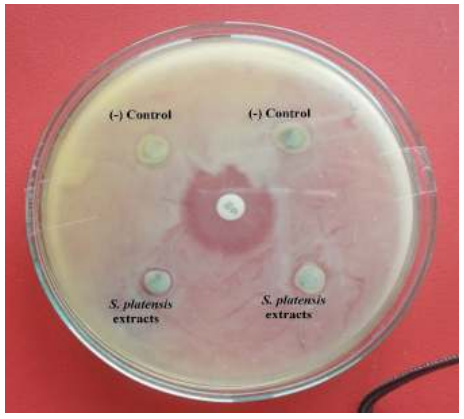


Figure 6: Antibacterial activities of gentamicin (+ control), deionised water (– control), and *S. platensis* extracts against *Proteus vulgaris*.

microbial population. Further investigations would involve covering the potency of *S. platensis* to synthesise silver nanoparticles.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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