Research Paper

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Phycosynthesized Silver Nanoparticles and Their Antibacterial Effect

Botany

KEYWORDS : *Spirulina platensis*, AgNPs, TEM, antibacterial effect

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ABSTRACT

Spirulina platensis plays a vital role as protein supplement and natural medicine due to its antioxidant and antimicrobial properties. The most efficient strain of Spirulina platensis was selected as CCC 477 suitable for biosynthesis of silver nanoparticles on the basis of reddish brown colour of the AgNPs synthesized solution with their characteristic peak identified by UV-Vis Spectroscopy. Further characterization regarding crystallanity, stability, morphology and size estimation was performed on the basis of XRD, FTIR, SEM and TEM analysis of biosynthesized silver nanoparticles. The antibacterial effect of Spirulina synthesized silver nanoparticles was examined against Gram positive as well as Gram negative pathogenic bacteria.

INTRODUCTION

Different routes for silver nanoparticle synthesis were applied as microwave synthesized AgNPs, laser mediated AgNPs synthesis , gas condensation method, thermal decomposition of silver compounds and biological reduction method. Chemical and physical methods are quite expensive and having toxic effects on biological systems therefore they are less favourable methods to synthesize nanoparticles**[1]**. Silver nanoparticles can produce excellent antibacterial effects on 16 bacterial species [2]. Silver ions acts as antibacterial agent against 650 types of diseases causing micro-organisms[3]. Interaction of *Spirulina platensis* with aqueous solution of silver nitrate ($AgNO_3$) and chloroauric acid was observed for the synthesis of silver, gold and bimetallic nanoparticles[4]. Present investigation deals with biosynthesis of silver nanoparticles using *Spirulina platensis* and its antibacterial effect against different pathogenic bacteria.

MATERIAL AND METHODS

Collection of *Spirulina platensis*

Spirulina platensis CCC 477 strain was brought to Spirulina Lab from IARI, New Delhi . *Spirulina platensis* was also collected from paddy fields of Dairy, Punjabi farm, Sikandarpur and Bichpuri.

Media Preparation

The media used for culturing S. *platensis* was CFTRI (Central Food and Technological Research Institute, Mysore) medium. The media used for culturing the bacterial strains was Nutrient Agar Medium (NAM) for *Staphylococcus aureus, Escherichia coli*, *Bacillus subtilis, Bacillus fusiformis* and *Bacillus licheniformis.*

Screening of different strains of *Spirulina platensis* for biosynthesis of AgNPs

Silver nanoparticles production was achieved by taking 1 g of wet biomass in a 250 ml conical flask with 100ml (10^{-3} M) aqueous $AgNO_3$ for different time intervals (2, 4, 6 days) incubated at room temperature. Theses flasks were shaken using orbital incubator shaker (at 150 rpm) for half an hour. A control unit was also maintained without algal extract. Silver nanoparticles synthesis was carried out by adding 1mM silver nitrate solution in the aqueous extract of *Spirulina platensis* incubated at room temperature.

Characterization of AgNPs [5]

UV-Vis spectral analysis

Synthesis of silver nanoparticles was confirmed by sampling the aqueous component of different time intervals and the absorption maxima was scanned by UV-Vis Spectrophotometer at the wavelength of 400-800 nm.

X-ray diffraction analysis

The XRD spectra was examined by thin film sample of $AgNO_3$ coated on glass plate. XRD analysis of biosynthesized AgNPs using *Spirulina* was carried out on living biomass on PANalyticalX' Pert Pro at AIRF, JNU, New Delhi operated with source of radiation as 45KV.The diffraction pattern was recorded by Cukα1 radiation with λ of 1.5406 Å in the region of 2θ from 20° to 80° at 2°/ min. with 2 sec. taken as constant time interval.

FTIR spectral analysis

FTIR spectroscopy is useful technique to recognize the existence of particular functional groups in the organic or inorganic compound**.** The bioreduced silver nitrate solution undergoes the process of centrifugation at 10,000 rotations per minute (duration 15 min) and the oven dried samples were grinded with KBr pellets used for FTIR analysis.

SEM analysis

Samples were prepared by drop coating the Ag nanoparticles solutions onto glass slides and further sputter-coated with gold. Secondary electron image (SEI) and backscattered electron image (BEI) modes were used. The films on the glass slides were allowed to dry prior to measurement.

TEM analysis

Particulate dimensions and structural analysis of lyophilized silver nanoparticles was examined by Transmission electron microscopy (TEM). Silver NPs suspended in distilled water were released upon the copper grids covered with carbon film and allowded to air dry in sterilized chamber before TEM analysis.

Particle size analysis

Particle size analysis was carried out using **Image J software** which is an free image analytic software launched by US NIH (National Institute of Health) extremely useful in the field of electron microscopy i.e. for sizing of SEM and TEM images[5].

Antibacterial assay of Silver nanoparticles

Paper Disc Diffusion Technique **[6]** was used to check antibacterial sensitivity of Spirulina synthesized silver nanoparticles. The results were recorded as Zone of Inhibition of bacterial growth as clear zone produced outside each disc with the help of rular in millimetre as –very high activity(11-15mm), High activity (7-10mm), Moderate activity (1-6mm) and No activity (-) [7].

RESULT & DISCUSSION

Isolation and Identification of *Spirulina platensis* strains

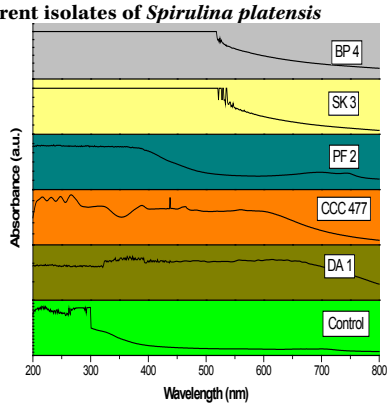
Algal strains of *Spirulina platensis* isolated from four different sites of Dayalbagh were abbreviated as DA 1, PF 2, SK 3 and BP 4. Algal colonies isolated from four different localities were puri-

fied using streak plate method in sterile condition using CFTRI medium incubated at 28°C.

Isolated strains (4) of *Spirulina platensis* were shown similar growth and morphological features with respect of CCC 477 strain. Identification of *Spirulina platensis* strains was done using traditional morphological approach, Geitler's conception [8,9].

Selection of the most efficient strain among living biomass of *Spirulina platensis* was done by taking UV-Vis spectra of all living cultures i.e. DA 1 (isolated from Dairy**),** CCC 477(IARI strain), PF 2 (isolated from Punjabi farm, Dayalbagh), SK 3 (isolated from Sikandarpur, Dayalbagh, and BP 4 (isolated from Bichpuri) incubated with 1mM $AgNO_3$ solution **. Figure 1** have shown the peculiar peak of AgNPs at 420nm in the UV-vis spectra of CCC 477 strain of *Spirulina platensis*. Present observation is in good agreement with biosynthesis of silver nanoparticles using *Spirulina platensis* biomass grown on BG-11 medium, had intense peak at 430nm with yellowish brown coloration of solution at 25°C and pH 7 [10].

Figure 1: Uv-Vis Spectra of biosynthesized AgNPs using different isolates of *Spirulina platensis*



The X-ray diffraction pattern of Ag-INPs i.e. silver nanoparticles synthesized via *Spirulina platensis* living biomass (CCC 477 strain) in **Figure 2** exhibits two characteristic peaks of silver metal at 38.32° and 44.54° which were indexed as the (111) and (200) Bragg reflections . Silver was compared with the JCPDS (Joint Committee for Powder Diffraction Standard) silver File No. 03-0921. Apart from these characteristic peaks responsible for silver nanoparticles, the XRD pattern shows some additional unassigned peaks, denoted with stars [11]. These peaks may be generated due to the formation of the crystalline bio-organic compounds or metalloproteins which were present in the aqueous component of Spirulina extract. The particle size estimated using Debye-Scherrer formula from FWHMs of 38.32° and 44.54° peaks were 30nm and 11 nm with average size AgNPs of 20.5 nm synthesized via living biomass of *Spirulina platensis*.

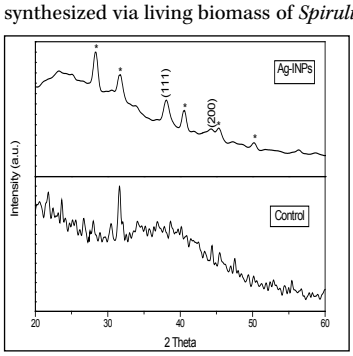


Figure 2: XRD micrograph of *Spirulina platensis* (CCC477) synthesized silver nanoparticles

An FTIR spectroscopic study was performed to delve the possible intermediary compound behind the formation of these nanoparticles and provide information about the functional groups. In the **Figure 3.1** 1543.10 and 1647.26 cm^{-1} peaks in the FTIR spectra of freeze dried KBr pellet refers to the amide II and amide I groups of proteins. Hydroxyl ion (OH-) stretching mode peaks were observed at 2968.55, 2773.73, 2958.90cm^{-1}. A very strong peak at 1383.01 cm^{-1} in all samples confirmed the presence of aromatic compound. FTIR profile of purified silver nanoparticles exhibited absorption peaks at 1242.20, 1383.01, 1647.26, 2958.90 cm^{-1} due to cyclic C-O, C=O and OH functional groups for living biomass (CCC477 strain) of *Spirulina platensis***. [Figure 3.2]** These obtained peaks were less intense with slight shifts when compared with control spectra of *Spirulina platensis***.**

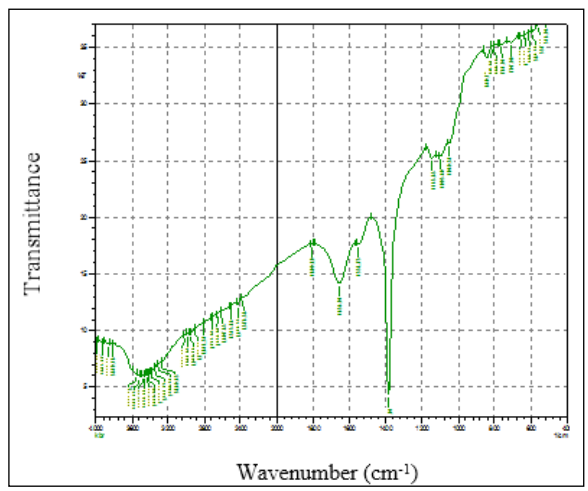


Figure 3.1: FTIR Spectra of *Spirulina platensis* (CCC 477 strain)

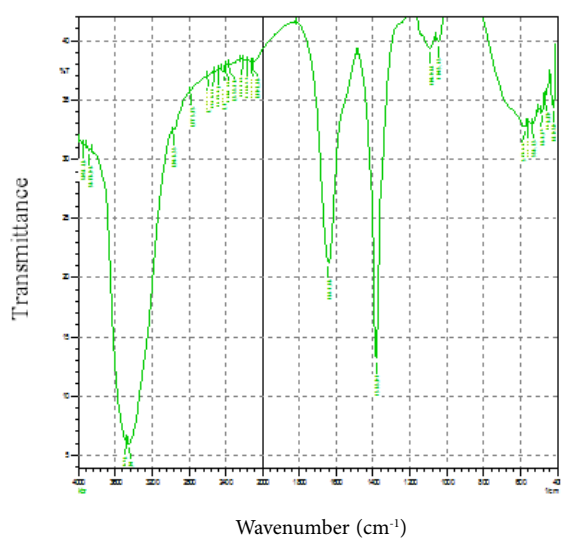


Figure 3.2: FTIR Spectra of Ag-INPs (*Spirulina platensis* CCC 477 synthesized silver nanoparticles)

SEM micrographs of Ag-INPs (AgNPs synthesized from living *Spirulina platensis* CCC 477 strain) in **Figure 4** explains the extracellular synthesis of silver nanoparticles along the coiled strands of *Spirulina platensis* with cubic, rectangular and spherical geometry **.** Most of the particles were monodisperse in nature with average size of 42nm but some of them get agglomerated with average size of 208 nm.

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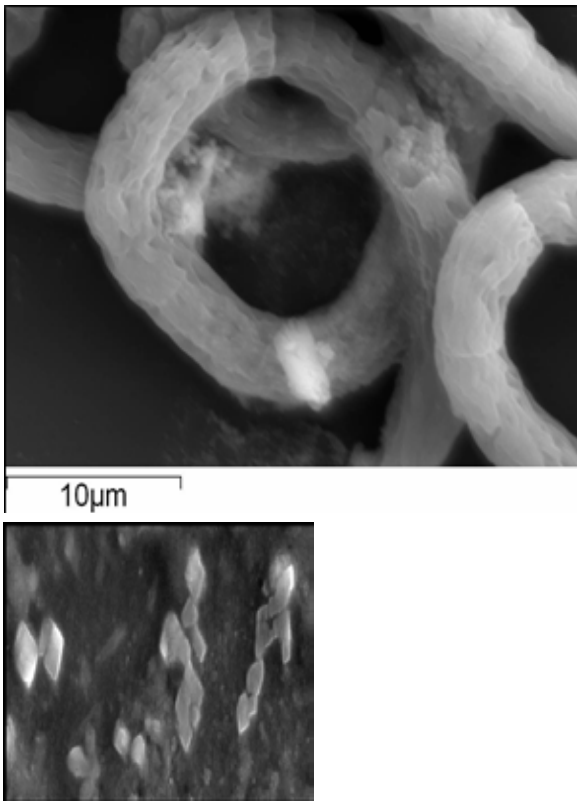

Figure 4: SEM micrograph of AgNPs impregnated in Spirulina platensis strands
AgNPs impregnated in *Spirulina platensis* strands
Crop image of silver nanoparticles

TEM analysis in **Figure 5** confirmed the presence of agglomerated small silver nanoparticles with mean diameters ranges from 2 to 26 nm synthesized by CCC 477 strain of *Spirulina platensis* .

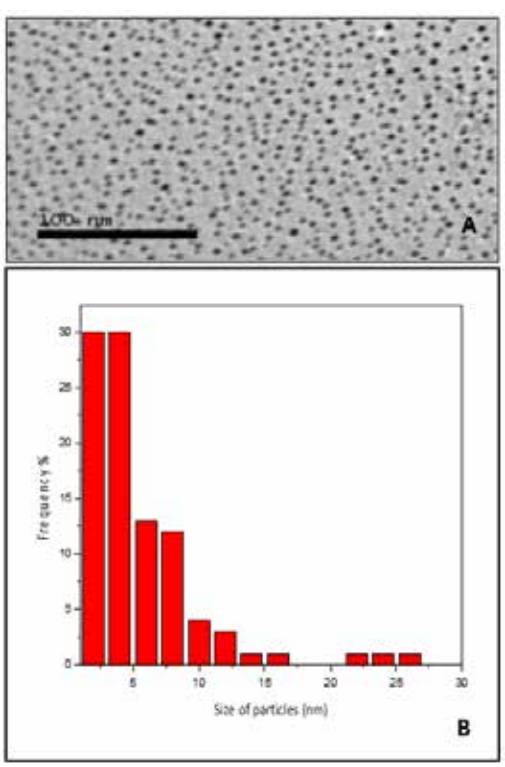

Figure 5 TEM Micrograph (A) and Particle Size Analysis (B) of Ag-INPs

Aqueous suspension of AgNPs was proved to be most effective against *E.coli* with ZI of 11.63 mm) at 100ppm conc. followed by 50 ppm concentration (ZI of 7.5mm) when compared with control sets (Chloramphenicol and $AgNO_3$ solution). Concentration of 100 ppm also exhibits moderate activity against *B.subtilis, B. fusiformis, and B.licheniformis* strain1 and low activity against *S.aureus* (5.0 mm) , *B.licheniformis* strain 2 (ZI of 6.5 mm) mentioned in **Figure 6** MIC of 25 ppm concentration was proved to be inhibitory against *B.fusiformis and B.licheniformis* strain 2 with Inhibition Zones of 3.38 and 4.25mm respectively as observed in **Figure 7.**

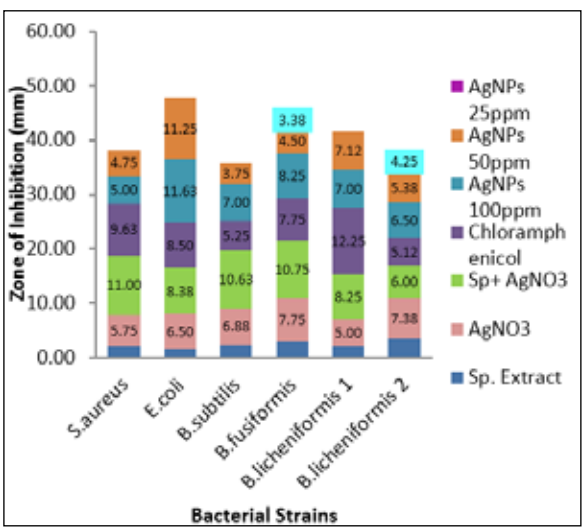

Figure 6: Antibacterial effect of Ag-INPs (*Spirulina platensis* CCC 477 strain synthesized AgNPs)

Synthesis of AgNPs via *Spirulina platensis*, characterization using UV-vis spectroscopy, XRD, FTIR and SEM analysis, toxicity of silver nanoparticles against *E.coli* was in accordance with the research findings of Mala *et al.*,2010[12]**.**

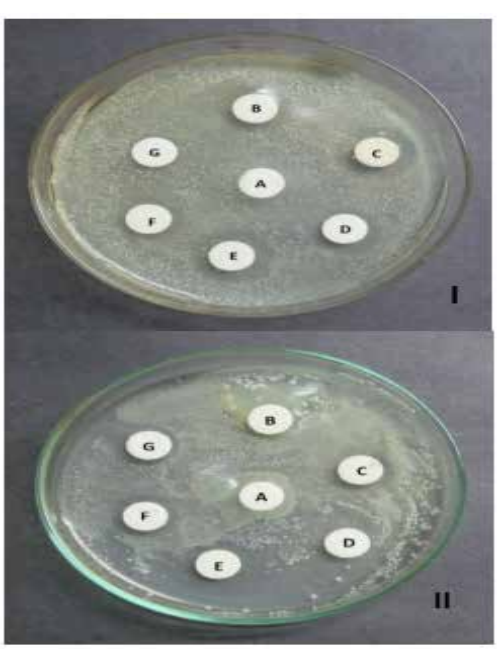

Figure 7- MIC of Ag-INPs against B. fusiformis and B. li-

cheniformis strain 2 A-Sp. extract B-AgNO3 C-AgNO3 + Sp. extract D-Chloram-phenicol E-100 ppm F-50 ppm G-25 ppm

CONCLUSION

Spirulina platensis (CCC 477strain) was able to produce antibiotic like substances or replace them was possible due to biosynthesis of silver nanoparticles which exerts a significant antibacterial effect against pathogenic bacterial species, responsible for infectious diseases in human and animals.

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