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GREEN SYNTHESIS OF SILVER NANOPARTICLES USING SINGLE CELL PROTEIN OF *SPIRULINA PLATENSIS*

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ABSTRACT

Spirulina platensis, a blue green microalga (cyanobacteria) is used as biologically active food additive and its therapeutic potential is well known, but its property of bioreduction of inorganic material is yet to be exploited.An aqueous solution of silver nitrate was treated with Spirulina powder for the formation of silver nanoparticles. The synthesis of nanoparticles was confirmed by change in color from pale green to reddish brown and Uv-vis absorption peak at 432nm. The size and shape of the nanoparticles were characterized by XRD, FTIR and SEM. The size of nanoparticles was confirmed as 12.77nm using X-ray diffraction analysis.

KEYWORDS: ***Spirulina platensis* , AgNPs, Uv-vis absorption ,XRD, SEM, FTIR.**

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INTRODUCTION

Nano' means very small in the range of 10−9 to be precise 10 to 100 nm in size. So nano materials in the broadest sense include anything composed of very small particles, particles in which a large fraction of the atoms sit close to the surface. Such particles are ubiquitous on the Earth's surface. We pay to have them, for example in electronic devices fabricated by nanotechnology. Nanoparticles occur as dust in the air, as small suspended colloids that make river water murky, in soil, in volcanic ash, in our bodies, and in technological applications ranging from ultra-tough ceramics to microelectronics. Nanoparticles both pollute our environment and help to keep the environment clean. Nano materials are having large surface areas with different dimensions from ten to a few thousand atomic diameters. Further, these properties are more important in various fields like Biological, chemistry, physics, materials science, engineering, soil science, environmental science, and geology[1]. Nanotechnology involving synthesis and applications of nanomaterials is a rapidly growing field with significant applications in various areas [2]. The attraction of silver nanoparticles (AgNPs) is mainly because of its application in therapeutics, biomolecular detection, catalysis and also as antimicrobial agents [3-6].

A vital aspect of nanotechnology concerns the growth of experimental processes for the synthesis of nanoparticles of different sizes, shape and proscribed dispersity. With the expansion of new chemical and physical methods, the concern for environmental contaminations are also sharp as the chemical methods concerned in the synthesis of nanoparticles make a large amount of hazardous by products. Thus, there is a need for microbe mediated synthesis that includes a clean, nontoxic and ecofriendly method of nanoparticle synthesis[7]. As a report, researchers in the field of nanoparticles synthesis and congregation have twisted to biological system of inspiration [8]. Nanoparticles demonstrate completely novel or improved properties compared with larger particles of the size and these new properties are derivative due to the variation in specific exact characteristics such as size, distribution and morphology of the particles [9]. It is well well-known to facilitate silver is an effective antibacterial agent and possesses a strong antibacterial activity against bacteria, viruses and fungi, even though the mechanism and the way of action are still not well known [10, 11]. Concerning the biological application of nanoparticles it has been emphasized that methods of biological synthesis through microorganisms including bacteria, yeasts, fungi and diatoms synthesizing inorganic materials either intra or extracellularly would make the nano particles more biocompatible. Various species of cyanobacteria and algae have been known to adsorb and take up heavy metal ions. Spirulina is gaining more attention in the field of medical science because of its nutraceutical and pharmaceutical importance [12]. In this study, we report the use of the Single Cell Protein of *Spirulina platensis* for the biosynthesis of pure metallic silver nano particles.

MATERIALS AND METHODS

(i) Spirulina platensis

Non living strains of *Spirulina platensis* were collected from Jalmahal (Jaipur), Ramgarh (Jaipur) and Rajkot (Gujarat) and Dairy (Dayalbagh).

(ii) Screening of different strains of Spirulina platensis for biosynthesis of AgNPs.

Non living strains of *Spirulina platensis* from Jalmahal (Jaipur), Ramgarh (Jaipur),Rajkot (Gujrat) and Dairy(Dayalbagh) were screened on the basis of synthesis of AgNPs. Silver nanoparticles synthesis using powder of *Spirulina platensis* was carried out by taking 5 mg of *Spirulina* powder in 50 ml of aqueous $AgNO_3$ solution of 10^{-3} molar concentration at room temperature similarly 5 mg of Spirulina powder in distilled water was kept as control [13]. Theses flasks were shaken at a rotation rate of 150 rpm for 1 hour. Thereafter solutions were filtered using Whatman filter

paper no.1 (Dia: 125mm).Silver nitrate was purchased from A.B. Company, Agra, India. Silver nanoparticles formations were carried out by taking 3 ml of aqueous *Spirulina* extract and 7 ml of silver nitrate solution of different molarities solutions were prepared as 1mM, 2mM, 3mM, 4mM, 5mM for 1-7 days of incubation period. The flasks were then incubated in the dark (to minimize the photo oxidation of silver nitrate), at room temperature. A control setup was also maintained without algal extract. The silver NPs solution thus obtained was purified by repeated centrifugation at 10,000 rpm for 15 min followed by re-dispersion of the pellet in deionized water. Then, the silver NPs were freeze dried.

(iii) Characterization of silver nanoparticles

UV-Vis spectral analysis

Synthesized silver nanoparticles was confirmed by sampling the aqueous component of different time intervals and the absorption maxima was scanned by UV-Vis spectrophotometer (SYSTRONICS-2203) at the wavelength of 400-800 nm. The bio-reduction of $AgNO_3$ ions in solution were monitored by periodic sampling of aliquots of aqueous component and measuring UV-vis spectra of the solution.

X-ray diffraction studies

The formation and quality of compounds were checked by X-ray diffraction (XRD) spectrum. The XRD pattern was measured by drop coated films of $AgNO_3$ on glass plate and carried out on BRUKER D8- ADVANCE. The diffraction pattern was recorded by Cu-kα1 radiation with λ of 1.5406Aº in the region of 2θ from 20º to 80º at 2º/ min. and the time constant was 2 sec.

FTIR spectral analysis

The bioreduced silver nitrate solution was centrifuged at 10,000 rpm for 15 min and the dried samples were grinded with KBr pellets used for FTIR measurements. The spectrum was recorded in the range of 4000 - 400 cm-1 using SHIMADZU 8400 operating in the diffuse reflectance mode at a resolution of 4 cm-1 in KBr pellets.

SEM analysis

JEOL JSM-5800 LV Scanning electron microscope at the National Institute of Oceanography, Goa, India was used. Samples were prepared by drop coating the Ag nanoparticles solutions onto glass slides and further sputter-coated with gold. Secondary electron image (SEI) backscattered electron image (BEI) modes were used. The films on the glass slides were allowed to dry prior to measurement [14].

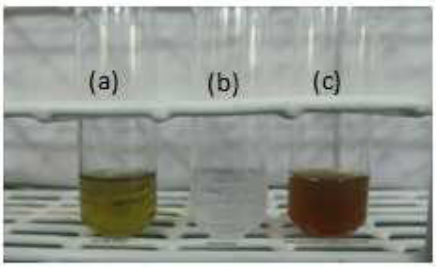
RESULTS AND DISCUSSION

Among non-living strains of *S. platensis* of Jalmahal Lake, Jaipur was found to show synthesis of silver nanoparticles as compare to other non-living strains (Ramgarh, Rajkot and Dairy strain). The colour of silver nitrate-algal extract changed from pale yellow to reddish brown after 2 days of incubation period at room temperature in 1mM $AgNO_3$ solution.(Fig1)

(c)

Figure 1

Biosynthesis of silver nanoparticles using Spirulina platensis



(a) Spirulina extract , (b) Silver nitrate solution, (c) Spirulina extract+$AgNO_3$ solution

Uv-spectra of 1mM, 2mM, 3mM, 4mM, 5mM solution was taken after 2 days which reveals the peak at 432 nm only in 1mM solution.(Fig2.) Metal nanoparticles have free electrons, which gives surface plasmon resonance (SPR) absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave [15]. The sharp bands of silver colloids were observed at 432 nm. The intensity of absorption band increases with increasing time period of aqueous component and consequent color changes were observed from greenish yellow color to reddish yellow. This characteristic color variation was due to the excitation of the surface plasmon resonance in the metal nanoparticles.

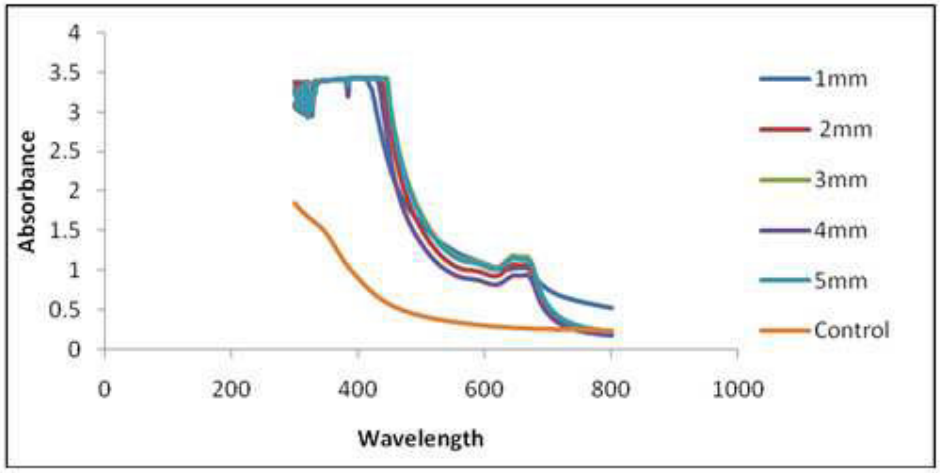


Figure 2
Uv-Vis spectra of silver nanoparticles synthesized by Spirulina platensis after 2 days of incubation period of different molarities solution

The XRD spectrum (Fig.3) showed three distinct diffraction peaks at 38.08°and 44.28° which are indexed the (111) and (200) of the cubic face-centered silver. The average grain size of the silver nanoparticles formed in the process was estimated from the Debye-Scherrer equation (d = (kλ×180)/ β Cos θβ Π) by determining the width of the (111) Bragg's reflection[16],where k is Scherrer constant, λ is the wavelength of the X-rays, β and θβ are full width half maximum of the Bragg angle, the estimated mean size of the particle was 12.77nm.

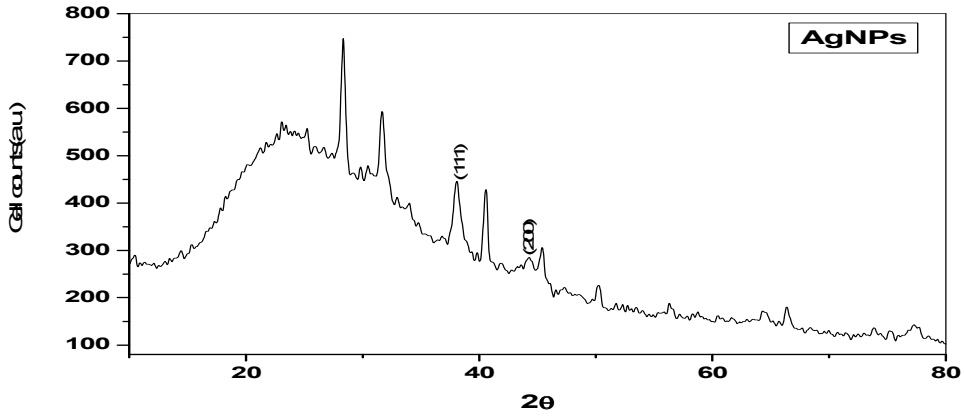


Figure 3
XRD pattern from drop-coated film of synthesized silver nanoparticles

FTIR spectroscopic studies are carried out to investigate the possible mechanism behind the formation of these nanoparticles and offer information regarding the functional groups [17]. The 1647.26 and 1543.10 peaks in the FTIR spectrum of freeze dried KBr pellet are characteristic of the amide I and amide II bands of proteins.The peak at 1143.83 cm^{-1} due to the phenolic group of Tyr residues was conspicuously absent in the FTIR spectrum of Spirulina powder after reaction with Ag ions. The very strong absorption peak at 1383.01 represents the presence of NO_2 which may be from $AgNO_3$ solution, the metal precursor involved in Ag nanoparticles synthesis process. Strong interaction of water with the surface of silver could be the reason for the OH- starching mode peaks at 2968.55, 2773.73, 2958.90 and OH in plane bending mode peaks at 1383. From the analysis of FTIR studies we confirmed that the carbonyl group from the amino acid residues and proteins has the stronger ability to bind metal indicating that the proteins could possibly from the metal nanoparticles (i.e., capping of silver nanoparticles) to prevent agglomeration and thereby stabilize the medium. This suggests that the biological molecules could possibly perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium[18].

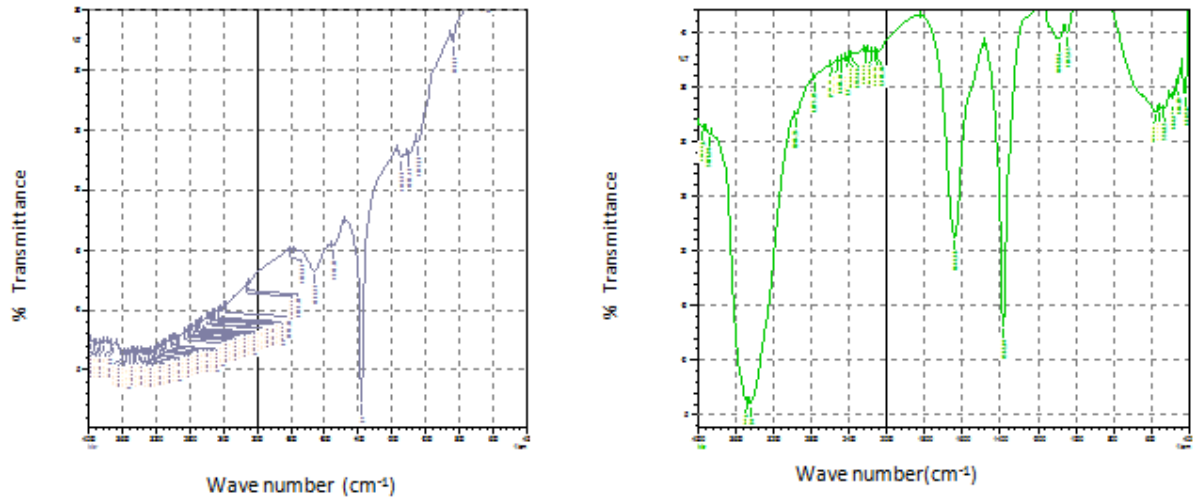


Figure 4
FTIR spectra of (a)Spirulina powder and (b) Spirulina synthesized silver nanoparticles

SEM analysis was carried out to understand the topology of silver NPs[19] which showed the biosynthesis of monodisperse spherical silver NPs and also aggregation of nanoparticles (Fig.5). The size and the distribution of the silver nanoparticles depend on the time of silver action. After one day of the silver ion action, large agglomerates of nanoparticles could be observed whereas after 5 days, the produced silver nanoparticles were relatively uniformly distributed along the surface of the cyanobacterium cells [20] which is in support with the present investigation.

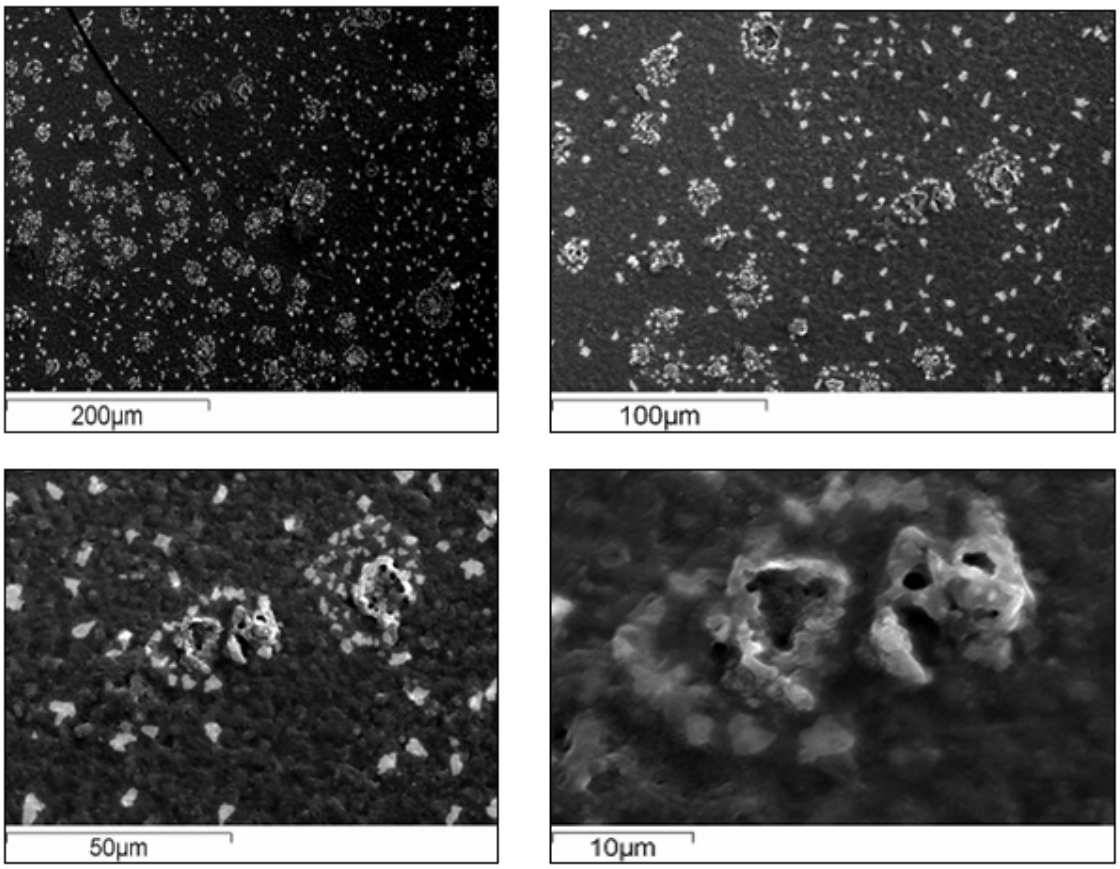


Figure 5
SEM images of Spirulina synthesized silver nanoparticles under different Magnifications(a)X250(b)X500(c)X1200(d)X3300

CONCLUSION

The rapid biological synthesis of silver nanoparticles using powder extract provides an environmental friendly, simple and efficient route for synthesis of benign nanoparticles. The average size of the silver nanoparticles was estimated as 12.77nm. From a technological point of view, these obtained silver nanoparticles have potential applications in the biomedical field and this simple procedure has several advantages such as cost-effectiveness, compatibility for medical and pharmaceutical applications as well as large scale commercial production.

REFERENCES

1. Thirumalai A , V. Prabhu D, Soniya M, Nanoparticle Synthesizing methods and its applications. Journal of Biosciences Research*, 1*(4): 259-270 (2010) .
2. Duran N , Priscyla D, Marcato P , Alves O, De Souza G , Esposito E,Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusarium oxysporum* species. Journal of Nanobiotechnology 3(8): 1-7 (2005).
3. Sadhasivam S, Shanmugam P , Kyu Sik Yun K, Biosynthesis of silver nanoparticles by *Streptomyces hygroscopicus* and antimicrobial activity against medically important pathogenic microorganism. Colloid and Surfaces B, 81(1): 358–362(2010).
4. Shrivastava S, Bera T, Singh S K , Singh G,Ramachandrarao Dash P,Characterization of antiplatelet

properties of silver nanoparticles. ACS Nano Biotech 3(6): 1357–1364, (2009).
5. Wei H, Chen C , Han B, Wang E ,Enzyme colorimetric assay using unmodified silver nanoparticles. Anal. Chem. 80(18) : 7051–7055,(2008).
6. Christopher P, Xin H, Linic S, Visible light enhanced catalytic oxidation reactions on plasmonic silver nanostructures. Nat Chem 3: 467–472 (2011).
7. Mukherjee P, Ahmad A, Mandal D, .Senapati S, Sainkar SR, Khan MI, Parishcha R, Ajaykumar PV, Alam M, Kumar R, Sastry M, Fungus-Mediated Synthesis of Silver Nanoparticles and Their Immobilization in the Mycelial Matrix: A Novel Biological Approach to Nanoparticle Synthesis Nano Letters, 1(10) : 515-519 (2001).
8. Udayasoorian C, Vinoth kumar K, Jayabalakrishnan R M, Extracellular synthesis of silver nanooparticles using leaf of Cassia auriculata, Digest Journal of Nanomaterials and Biostructures, 6(1): 279 – 283 (2011).
9. Mukunthan K S, Elumalai E K, Trupti N Patel , V Ramachandra Murty V, Catharanthus roseus: a natural source for the synthesis of silver nanoparticles Asian Pacific Journal of Tropical Biomedicine, 1(4) : 270-274 (2011).
10. Sharma VK, Yngard RA, LinY ,Silver nanoparticles: green synthesis and their antimicrobial activities, Adv. Colloid Interface Sci. 145(1-2): 83–96 (2009).
11. Krutyakov YA, Kudrinskiy AA, Olenin AY, Lisichkin GV, Synthesis and properties of silver nanoparticles: Advances and prospects, Russian Chem. Rev; 77(3):233– 257 (2008).
12. Mala R, Sivasankari M , Umadevi G ,Synthesis, Characterization and antimicrobial activity of silver nanomaterial against *E.coli*. International Journal of Nanoscience and Nanotechnology. 1(1): 49—56 (2010).
13. Govindaraju K, Basha SK, Kumar VG, Singaravelu G, Silver, gold and bimetallic nanoparticles production using single cell protein (*Spirulina platensis*). Journal of Material Science, 43(15): 5115 – 5122. (2008).
14. Rajesh S, Patric Raja D, Rathi JM , Sahayaraj K, Biosynthesis of silver nanoparticlesusingUlva fasciata(Delile)ethyl acetate extract and its activity against *Xanthomonas campestris pv. Malvacearum.* Bionanoparticle for Phytopathogen management, 5(Supp. Issue): 119-128 (2012).
15. Mallikarjunaa K, Narasimhab G, Dillipa GR, Praveenb B, Shreedharc B, Sree Lakshmic C, Reddyc BVS, Deva Prasad Rajua B, Green synthesis of silver nanoparticles using Ocimum leaf extract and their characterization, Digest Journal of Nanomaterials and Biostructures 6 (1): 181 – 186 (2011).
16. Borchert H, Shevchenko EV, Robert A, Mekis I, Kornowski A, Grubel G, Weller H, Determenation of nanocrystal sizes: a comparision of TEM, SAXS and XRD studies on highly monodisperse Co.Pt 3 particles. Langmuir 21(5): 1931 (2005).
17. Theivsanthi T, Alagar M , Electrolytic Synthesis and Characterizations of Silver Nanopowder . Nano Biomedicine and Engineering 4:58-65 (2012**)**.
18. Sathyavathi R , Krishna MB, Rao SV, Saritha R, Rao DN, Biosynthesis of silvernanoparticles using *Coriandrum sativum* leaf extract and their application in nonlinear optics Advanced Science Letters. 3,138-143 (2010).
19. Mani U, Dhanasingh S, Arunachalam R, Paul E, Shanmugam P, Rose C, Mandal AB , A simple and green method for the synthesis of silver nanoparticles using *Ricinus communis* leaf extract, Progress in Nanotechnology and Nanomaterials, 2 : 21-25 (2013)
20. Tsibakhashvili NY, Kirkesali EI,Pataraya DT, Manana AG, Kalabegishvili T L, Gvarjaladze DN, Tsertsvadze GI, Frontasyeva MV, Zinicovscaia I I, Wakstein MS, Sergey N. K, Shvindina NV, Shklover VY, Microbial Synthesis of Silver Nanoparticles by *Streptomyces Glaucus* and *Spirulina platensis*, Nanomaterials: Applications and Properties (NAP-2011). 2(2):306-310(2011).