SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES FROM *PSEUDOMONAS AURIGNOSA* MTCC 2297

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ABSTRACT

In this work the synthesis of stable silver nanoparticles by the bioreduction method from *Pseudomonas aurignosa* MTCC2297 is proposed. The culture supernatant of these nanoparticles by reduction of silver ions coming in contact with cell filtrate was fast and formed within minutes. The quantitative formation of nanoparticles is monitored by UV-V is Spectroscopy and showed a peak of 440-450 nm corresponding to the surface plasmon absorbance of nanoparticles. Further characterization was done using FTIR (Fourier Transform Infrared Spectroscopy), XRD (Xray Diffraction Analysis) and AFM (Atomic force Microscopy). FTIR spectroscopy used for quantitative anayses of the reaction products [4]. XRD of silver nanoparticles exhibited 2θ values corresponding to the silver nanocrysta [2]. AFM shows the morphology and measure size, shape of silver nanoparticles. The average size of the particle diameter was found to be 160-360 nm using AFM analysis.

Keywords: UV-V is Spectroscopy, FTIR (Fourier Transform Infrared Spectroscopy), XRD (X ray Diffraction Analysis) and AFM (Atomic force Microscopy).

I. INTRODUCTION

The term 'nano' is adapted from the Greek word meaning "Dwarf" when used as a prefix, it implies 10^{-9}. A nanoparticle is a microscopic particle with atleast one dimension less than 100 nm. Nanoparticles are of great scientific approach as they form the gap between bulk materials and molecular structures. And also, will increase miniaturization which becomes more important in areas such as computing, sensors and biomedical applications. For the synthesis of silver nanoparticles, biological method have an advancement over physical and chemical methods as it does not require high energy, pressure and toxic chemicals. Silver nanoparticles play a major role in the field of biology and medicine due to their physiochemical properties. Due to extraordinary antimicrobial properties of silver and the low toxicity of free silver ions, the interest for food applications of these silver nanoparticles is tremendously increasing [7]. However, the EU safety regulation which provides the rules that the presence of silver ions in food matrices and limits its amount to 0.05 mg Ag/kg must be satisfied. Nanomaterials shows considerably changed physical, chemical and biological properties compared to their macroscale counterparts. Gold, Silver and copper have been used mostly for the synthesis of stable dispersion of nanoparticles, which are used in the field of photonics, biological labeling, opto-electronics, and Surface Enhanced Raman Scattering (SERS) detection [1].

The growth of unwanted bacteria is still a problem for the food industry and in the medical field. Therefore, there is a need for methods to kill or slow down the growth of these bacteria. An interesting alternative method is the use of metallic nanoparticles is silver nanoparticles. In order to achieve an understanding of this effect, knowledge about the structure of bacteria is needed. In particular, the bacterial membrane and the contained proteins are of special interest, because the silver has to react with it in order to penetrate the bacteria. Silver has for a long time been known to be toxic to a wide range of bacteria, and this has been utilized in various applications. Silver compounds are used as preservative in a variety of products and in the medical field to treat burns and infections [5].

This paper report the biological synthesis of stabilized silver nanoparticles using cell filtrate from *Pseudomonas aurignosa* MTCC 2297, its characterization by using UV-Vis spectroscopy, FTIR (Fourier Transform Infrared Spectroscopy), XRD (Xray Diffraction Analysis), and AFM (Atomic force Microscopy). Further studies can be conducted to explore applications of the silver nano particles

prepared from the *Pseudomonas aurignosa* MTCC 2297.

II. MATERIALS

Silver nitrate ($AgNO_3$) and *Pseudomonas aurignosa* MTCC 2297 *was* purchased from Helini Biomolecules, Chennai.

III. CULTURE CONDITIONS

The *Pseudomonas aurignosa* MTCC 2297 was cultured on nutrient agar plates and pure colonies were isolated. The loop full of *Pseudomonas aurignosa* MTCC 2297 was inoculated into 100ml nutrient broth and incubated for 36 hours at 37°C. After incubation the content was filtered by whatmann filter paper No.I. After filtration the observed pH of cell filtrate was above 7. For The cell filtrate was centrifuged at 5000 rpm for 30 minutes for purification. The supernatant was thus obtained and collected. This supernatant was used as the starting material for synthesis of silver nanoparticles.

IV. SYNTHESIS OF NANOPARTICLES

Into these 100 Ml of culture supernatant, a carefully weighed quantity of silver nitrate was added to the Erlenmeyer flask to yield overall silver ions concentration and incubated the reaction under dark conditions for 3 days. The silver nanoparticles solution thus obtained was purified by repeated centrifugation at 5000 rpm for 30 minutes followed by redispersion of the pellets of silver nanoparticles into 10 ml of toluene. After freeze, purified silver nanoparticles were used for further analysis [9]. The formation of nanoparticles is observed by visual change in colour from colourless to reddish brown. Formation of reddish brown is due to the surface plasmon resonance property of silver nanoparticles. The bioreduction of silver ions was monitored by measuring the UV-V is spectra of the reaction medium at equal time intervals followed by UV-Vis spectrophotometer at a resolution of 1 nm. This is further confirmed by FTIR, XRD, and AFM.

V. CHARACTERIZATION OF NANOPARTICLES

UV-V is absorbption spectrum was recorded after the nanoparticle formation, provided surface plasmon resonance exists for the metal nanoparticles. By using FTIR analysis interaction between protein and the silver nanoparticles was analysed and also evaluated functional groups that might be involved in formation of nanoparticles. The XRD spectra were taken with an X-Ray diffractometer at room temperature using Cu K α radiation $\lambda = 1.5406$ A over a wide range of Bragg angles $(10° < 2\theta < 80°)$.

The size of the nanoparticles was calculated through the Scherer's equation.

$$D = K\lambda/\beta \cos \beta,$$

Where D is the average crystal size, K is the scherer coefficient (0.89), λ is the wavelength, θ is Bragg's angle (2θ), β the full width at half maximum (FHWM) in degrees [6]. A thin film of the sample was prepared on a glass slide by dropping 100 μ l of the sample on the slide, and was allowed to dry for 5 min. The slides were then scanned with the AFM. Three dimensional topography and morphological study of biosynthesized silver nanoparticles were observed by AFM.

VI. RESULTS & DISCUSSION

A comprehensive study of Extracellular synthesis, stabilization and characterization of Silver nanoparticles from *Pseudomonas aurignosa* MTCC2297 was carried out in this work.

A. UV-V is Spectroscopic Analysis

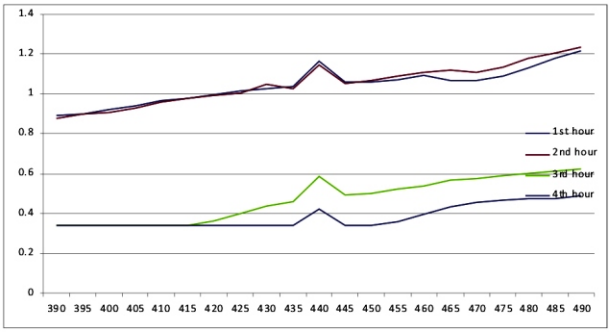

Fig. 1. UV Visible spectra for *Pseudomonos aurignosa* MTCC 2297

Formation of silver particles by reduction with silver nitrate using *Pseudomonos aurignosa* MTCC 2297 extract samples were characterized using UV-V is Spectroscopy and this is used for the analysis of silver nanoparticles. Finally the reaction was kept about for a month in the room temperature. The solution remains stable without aggregation of particles. Figure:

1 depict the UV-V is spectrum, a graph drawn between Wavelength in nm versus Absorbance. The Figure: 1 also shows the strong surface plasmon centered at 435-440 nm, which indicates the silver nanoparticles formation [7].

B. FTIR Analysis

This analysis was carried out to identify possible biomolecules and cell metal ions interaction for formation and stabilization of silver nanoparticles. The results of FT-IR analysis is presented in Figure: 2 (a) shows before adding silver nitrate and Figure: 2 (b) after synthesis of nanoparticles. Figure: 2 (a) showed the FTIR Spectrum that did not contains silver nitrate where as Figure: 2 (b) showed spectrum of extract containing silver nitrate. Spectra Figure: 2 (a) showed transmission peaks at 3256.41, 1635.80, 526.17, and 509.33 cm^{-1}. Similarly, transmission peaks for the cell extract containing silver nanoparticles were obtained at 3263.82, 1632.37, 523.08, and 506.56 cm^{-1}. Two absorption peaks located around 523.08 and 506.56 cm^{-1} can be assigned as the absorption of *– C– O– C*. The adsorption at around 1632.37 were attributed to stretching vibration of carboxyl group (*– C = O*). The broad and strong band at 3263.82 is due to bounded hydroxyl (– OH) or amine groups (–NH) of *Pseudomonos aurignosa* MTCC 2297 extract. The carboxylgroup at 1635.80 cm^{-1} was shifted to 1632.37 cm^{-1}. The results indicates that hydroxyl (–OH) carboxyl group (*– C = O*), amine groups (–NH) are mainly involved in fabrication of silver nanoparticles [3] [8].

C. X-Ray Diffraction

The crystalline natureof silver nanoparticles was obtained from the analysis of the X-Ray diffraction (XRD) pattern. The pattern was then analyzed and the FWHM was used with the scherrer's formula to determine mean particle size of the biosynthesized silver nanoparticles. The intensive diffraction peak at a 2θ value of 28.24 from the (111) plane of face centered cubic (fcc) silver unequivocally indicates that the particles are made of pure silver. Three additional bands are observed at 31.8, 47.7, 54.79 they

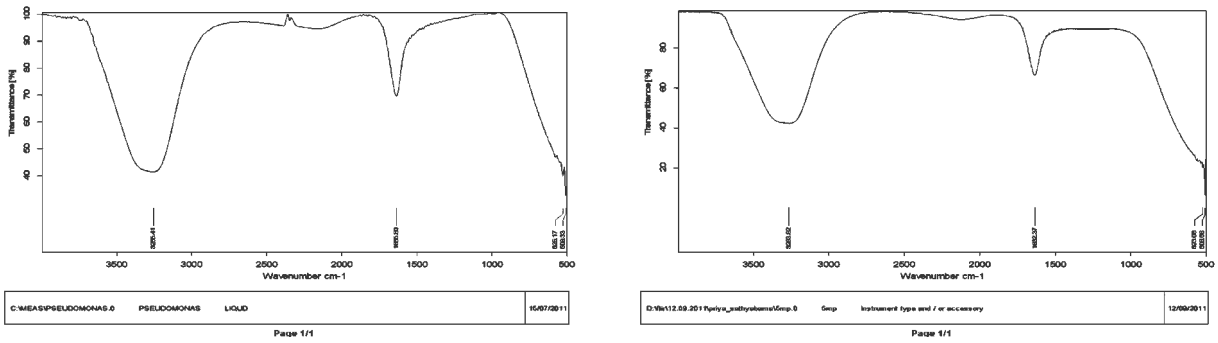

Fig. 2. FTIR spectrum of (a) Plain *Pseudomonos aurignosa* MTCC 2297 extract and (b) silver nanoparticles after synthesized using *Pseudomonos aurignosa* MTCC 2297

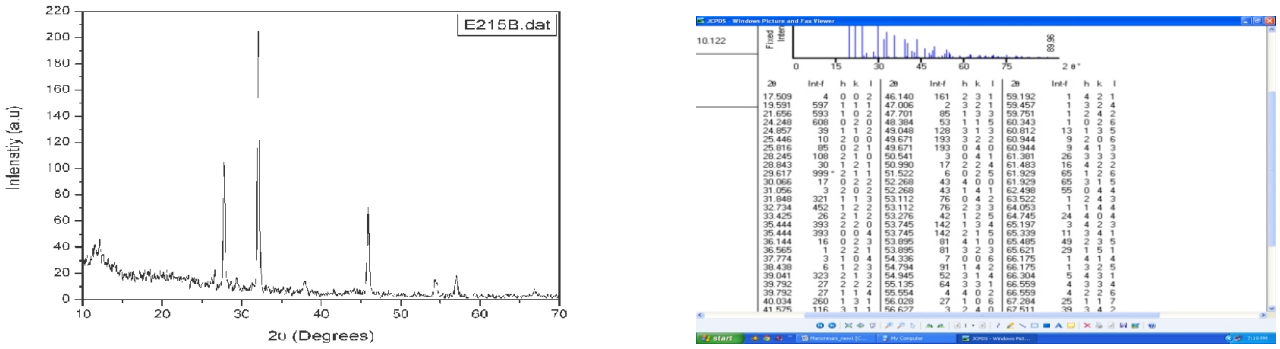

Fig. 3. (a) X-Ray diffraction pattern of synthesized silver nanoparticles from extract of *Pseudomonos aurignosa* MTCC 2297, (b) X-ray Diffraction peak List of Silver Nanoparticles(JCPDS file)

correspond to the (200), (220) and (311) planes of silver respectively. Figure: 3 (b) explains the X-ray diffraction peak list of silver nanoparticles. In the obtained spectrum, the Bragg peak position and their intensities were compared with the standard JCPDS files. The results shows that the particles have a cubic structure [5] [6].

D. Atomic Force Microscopy (AFM)

A thin film of the sample was prepared on a glass slide by dropping 100 µ l of the sample on the slide, and was allowed to dry for 5 min. The slides were then scanned with the AFM. The focus of the present study was to investigate the structural and surface alterations induced in *Pseudomonos aurignosa* MTCC 2297 by the exposure of silver nanoparticles using AFM. It is expected to obtain easily achievable images by AFM with different orientations in space and accurate measurements [9]. Atomic Force Microscopy has provided further insight in the morphology and size details of the silver nanoparticles. A representative AFM image recorded from the silver nanoparticles. Figure: 4 shows the particles are spherical in shape, and polydispersed in nature under optimized condition for the production of silver nanoparticles. Hence the size of the silver nanoparticles was measured in the range of 160-360 nm. A minimum of six images for the sample were obtained using AFM technique and analyzed to ensure reproducible results.

The topographical image of irregular silver nanoparticles is reported in Figure 5. Silver nanoparticles formation and its morphology were clearly observed in below figure.

IV. CONCLUSION

Silver ions were biologically reduced to metallic silver nanoparticles by mediation of *Pseudomonos aurignosa* MTCC 2297 extract provides an eco friendly, simple and synthetic route for synthesis of stabilized silver nanoparticles. The bioreduced silver nanoparticles were characterized using UV-V is spectroscopy, FTIR, XRD and AFM techniques. The formation of the silver nanoparticles was confirmed by visual observations of changing of solution from colourless to reddish brown

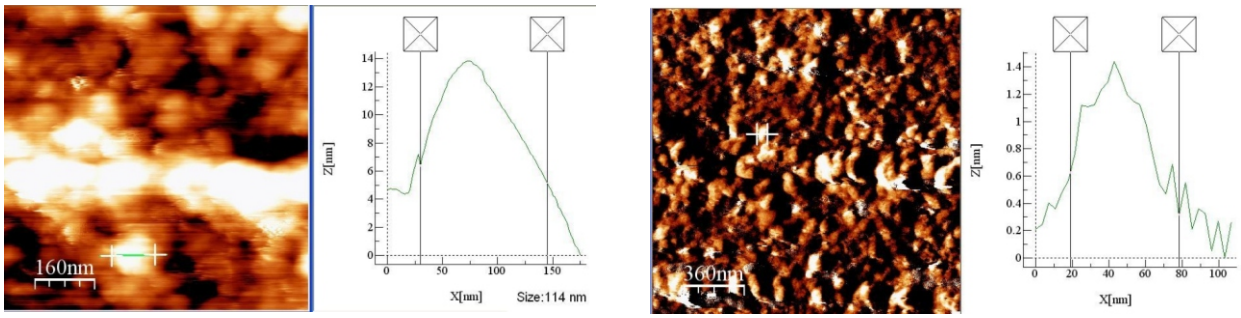

Fig. 4. AFM picture of the sample

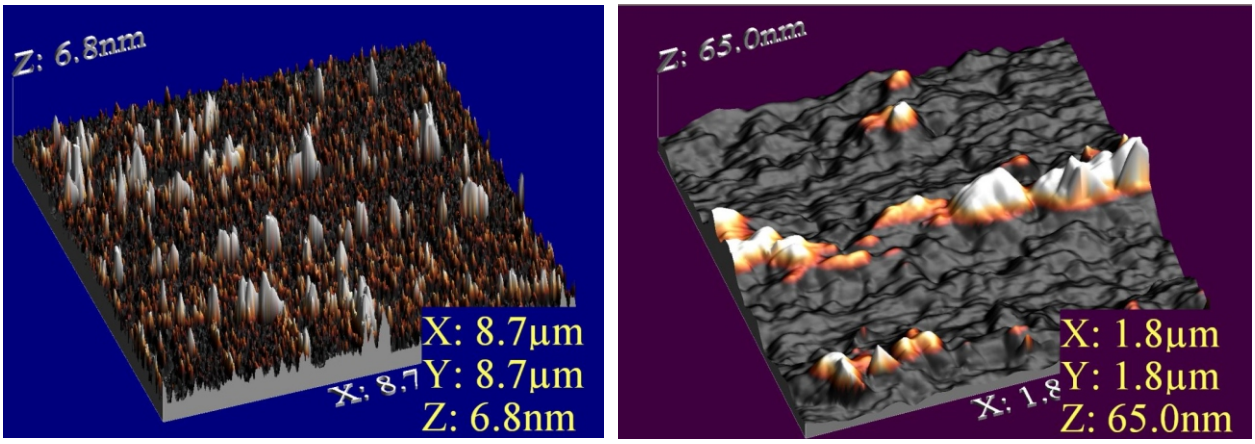

Fig. 5. AFM shows the three dimensional images of Silver nanoparticles

as well as a prominent peak found at 440-445 nm. The size of the silver nanoparticles was measured in the range of 160-360 nm by AFM analysis. Results conclude that isolated *Pseudomonos aurignosa* MTCC 2297 is a distinct producer of silver nanoparticles and have shown ability to reduce metal ions to form metallic nanoparticles. More studies could be further made by using SEM (Scanning Electron Microscopy) and TEM (Transmission Electron Microscopy) Analysis for optimizing the biosynthesis reaction and also for the biosynthesized silver nanoparticles application in food systems as preservatives. Silver nanoparticles could provide a safer alternative to conventional antimicrobial and antibacterial agents [5] [10].

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VI. ACKNOWLEDGEMENT

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