Original Article

Microbial Synthesis of Silver Nanoparticles by *Actinotalea sp.* MTCC 10637

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ABSTRACT

Silver nanoparticles were synthesized by a bacterial isolate, retrieved from soil which was identified as *Agrococcus sp.* The bacterial culture was isolate by enrichment culture technique using $AgNO_3$ in LB medium. Silver nanoparticles were characterized by using transmission electron microscopy. It was observed that bacterial isolate produced silver nanoparticles in size ranged of 5-80 nm. Particle size analyzers gave the average diameter of silver nanoparticles as 555.1. Further, by using UV-Visible spectrophotometer culture broth showed silver surface Plasmon band at 311nm. FTIR (Fourier transform infrared spectroscopy) analysis showed four major peaks at 3450.47 cm^{-1}, 2077.67 cm^{-1}, 1636.91 cm^{-1} and 1017.70 cm^{-1}.

Keywords: Bacteria, Nanoparticles, *Actinotalea,* Silver, Nanobiotechnology.

INTRODUCTION

The word "Nano" is originated from the Greek word "Dwarf". It means "a billionth." A nanometer is a billionth of a meter, that is, about 1/80,000 of the diameter of a human hair, or 10 times the diameter of a hydrogen atom. Nanoparticles are defined as particles less than 100nm in diameter that exhibit new or enhanced size dependent properties compared with larger particles of the same material. This technology has been used in medicine which includes drug carrier, cell dye, cell separation, clinical diagnosis and disinfection Because of large number of problems associated with physical and chemical synthesis method for SNPS, there is a great demand to develop eco-friendly processes for the synthesis of nanoparticles using microorganisms. Green synthesis of Ag nanoparticles by bacterial isolate involves three main steps: 1) Isolation of bacterial culture from environment sources. 2) Formulation of

growth medium 3) Optimization of culture condition for synthesis of SNP is by selected isolates. Microorganisms have the ability to grow in extreme of environment, which is enriched in heavy metals. Due to their tolerance to toxic chemical/metals, microorganisms have developed a number of mechanisms by which they can detoxify them and result in production of nanoparticles of specific size and shape by highly regulated mechanism.

By use of bacterial isolates from soil, silver nanoparticles have been synthesized by number of investigators. Bacteria are omnipresent in soil and easy to isolate from environmental sources such as soil and water by using selective growth medium. Silver nanoparticles as nanoparticle production possesses more surface atoms than a micro particle, which greatly improves the particle's physical & chemical characteristics[1]. The application of silver nanoparticles, when they interact or incorporate with other material such as polymer, tio_2 etc is being reported by investigators earlier[2]. The synthesis and assembly of nanoparticles would benefit from the development of clean, nontoxic and environmentally acceptable ''green chemistry'' procedures, probably involving organisms ranging from bacteria to fungi and even plants[3,4]. Scientists have reported the basic steps for metal nanoparticle biosynthesis and classified them either intracellular or extra cellular biosynthesis[5]. The advantages of extra cellular biosynthesis over intracellular production and also have been reported the main factor such as NADH-dependent reductase and Cytochrome C enzyme which are responsible of bioreduction processes in nanometal production. There are several reports where in the soil isolates have been used for synthesis of metal nanoparticles[6,7]. Earlier investigators have reported the formation of SNP_S by bacterial &Actinomycetes[8-11]. Synthesis of nano-particles from gram positive bacteria was also reported by researchers[12].

Many investigators have reported the characterization of microbial synthesized nanoparticles by *Penicillium* strain[6] and by *Aspergillus Niger*[13].

EXPERIMENTAL DETAILS

Isolation

The bacterial culture was isolated from soil of vicinity of jeweler market Hissar. Bacteria were grown on Luria broth agar medium (Hi-Media and Sisco Research Laboratory) Containing 5 mM $AgNO_3$ (S.d. Fine chem. Ltd.) under aerobic conditions at 30 ^{0}C. The pure isolates were preserved at -80^{0}C in culture medium containing 70 %(v/v) glycerol.

Characterization of isolates

The pure isolates obtained were characterized in terms of their morphological, physiological and biochemical nature from IMTECH. CHANDIGARH.

Bio synthesis of silver nanoparticles

The 50 ml of Luria broth was inoculated with a loopful of bacteria containing 5 mM $AgNO_3$and incubated for a period of 5 days in darkness at room temperature. Upon visual observation, the cultures incubated in the presence of silver nitrate showed a colour change from yellow to brown.

Characterization of bioreduced silver nanoparticles

UV-Vis- Spectroscopy (ultra violet visible spectroscopy) (SHIMADZU UV-2450 Sr. No. A10834701961): UV-Vis-Spectroscopy measurements were carried out as a function of time of the reaction at room temperature. Sample of 1ml withdrawn from silver nitrate solution

incubated with *bacteria* by drawing 1cm^3 of the sample and the absorbance was recorded at a resolution of 1nm at 300-600nm using UV-VIS spectrophotometer.

F.T.I.R. (Fourier Transform Infrared Spectroscopy) Measurements: (Spectrum BX Parkin Elmer Spectrum BXII)**:** The KBr pellets formed using Hydraulic Pelette Press (Kimaya Engrs). Put 0.1µl of incubated bacterial broth culture in center of KBr pellet.

PSA (Particle Size Measurement:. (Zetasizer Ver. 6.01(Malvern) MAL1039244)**:** Particle sizing experiments were carried out by means of laser diffractometry, using Zetasizer instrument (Malvern), equipped with Hydro dispersing unit (Malvern).We take the measurements in the range between 0.1 and 1000 µm.

TEM (Transmission Electron Microscopy) (HITACHI H7650): Samples were prepared for TEM analysis by separating the biomass from the liquor by centrifugation and washed twice in sterile distilled water. The samples were fixing for 24 hour at 4^{0}c in 2.5% glutaraldehyde in 0.1 M sodium Cacodylate buffer (pH7.4) and followed by three washes of 15 minute in 0.1 M sodium Cacodylate buffer. After fixation, the cells were sediment (1500 rpm, 10 min) and washed thrice with distilled water .After a second fixation step of 2 h at 4^{0}c in 1% osmium tetra oxide, the cells washed in distilled water and then stain with 1% Uranyl acetate in 25% ethanol for 1hour. The pellet was subject to dehydration with 30%, 50%, 70%, 90% and 100% ethanol for 20 min at each concentration followed by two changes in absolute ethanol. Since ethanol does not possess good miscibility with spur resins, propylene oxide was use as a linking agent. The dehydrated pellet was kept in propylene oxide for 15 min following which the infiltration of the resin was done by placing the pellet in a 3 : 1,2 : 2 and 1 : 3 mixture of propylene oxide and spur resin for 30min. and in pure spur resin for overnight at room temperature. Embedding was carrying out using pure spur resin in BEEM capsule. Polymerization was carrying out at 60°C for three days. Ultrathin sections were cut using an ultra microtome and taken on copper TEM grids (40 mm ´ 40 mm mesh size). The sections were slightly stain with Uranyl acetate for 5 minute and lead citrate for 2 minute to TEM analysis Sections were mounted on cu grids. Micrographs were taken with a microscope (HITACHI) at 100 kV instrument.

RESULTS AND DISCUSSION

Isolation and characterization of strain capable of synthesizing silver nanoparticles

A large number of bacterial colonies appeared on LB medium containing $AgNo_3$.Most of colonies of brown color with different shapes and sizes. (Fig.1)This suggests that soil sample is already enriched with bacteria, which have ability to grow in silver containing media.

Among 20 pure isolates only one was characterize on the basis of morphological, physiological and biochemical features.

The isolated bacteria were identified as *Actinotalea sp.* through their morphological, physiological and biochemical tests (Bagnara *et al*. 1985).

Synthesis of silver nanoparticles

Bacteria was grown in LB broth containing $AgNo_3$ (5.0mM).Synthesis of SNP_S could be observed with the appearance of brown coloration in the medium. (Fig.2).

Characterization of bioreduced silver nanoparticles

UV-Vis- Spectroscopy Analysis: The UV-Vis spectra recorded from the reaction medium after 5 days (Fig.3). Absorption spectra of silver nanoparticles formed in the reaction media has absorbance peak at 311 nm.

FTIR Spectral Analysis: FTIR spectrum of silver nanoparticles synthesized by bacterial culture after 5 days was shown below. The bands seen at 3450.47cm−1 and 2077.67 cm−1 correspond to the stretching vibrations of primary and secondary amines, respectively; while their corresponding bending vibrations were seen at 1636 .91cm−1.

The band observed at 1017.70cm−1 can be assigned to the C–N stretching vibrations of aromatic and aliphatic amines, respectively. (Fig. 4).

Particle Size Determination: Laser diffraction revealed that particles obtained are polydispersity mixture with the size ranging between hundreds on nanometres and micrometers (Fig. 5). The average diameter of the particles found to be 555.1 nm for (S_3) .The two peaks observed in the result, which tell the diameter of SNP_S for s_3 2448nm, 401.7nm and peak intensity found to be 89.7%, 10.3%, respectively.

TEM Analysis: Micrographs were taken with a microscope (HITACHI) at 100 kV instrument. (Fig. 6).

CONCLUSION

In summary, from this study it can be concluded that the bio-reduction method is a good alternative to produce nanoparticles than the electrochemical methods. Especially, extracellular synthesis offers the advantage of obtaining significant quantities in relatively pure state and can easily be processed by filtering the cells and isolating the particles through cell-free filtrate. The development of eco-friendly process for the synthesis of metallic nanoparticles is an important step in the field of application of nanotechnology to new technologies especially in chemistry, electronics, medicine and biotechnology. Further studies are required to optimize, the size of SNP_S, so that they can be used in nanoparticulate form.

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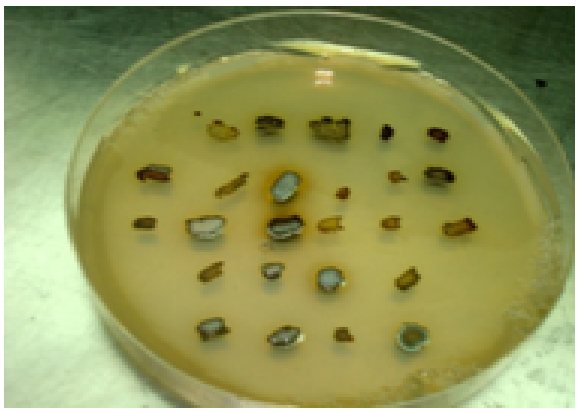

Figure 1. Growth of purified bacterial cultures on LBA plates containing $AgNO_3$

Figure 2. Appearance of brown color from off white indicates the formation of SNPs

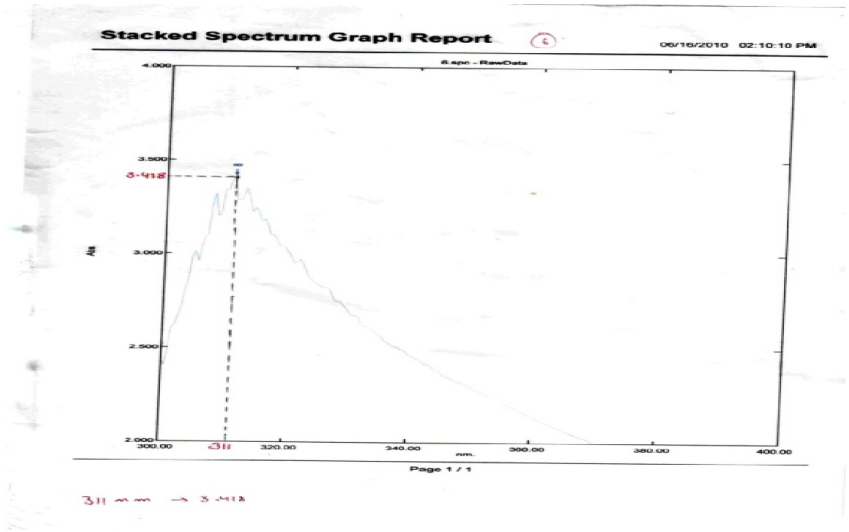

Figure 3. UV-Vis- Spectroscopy Analysis of bioreduced silver nanoparticles

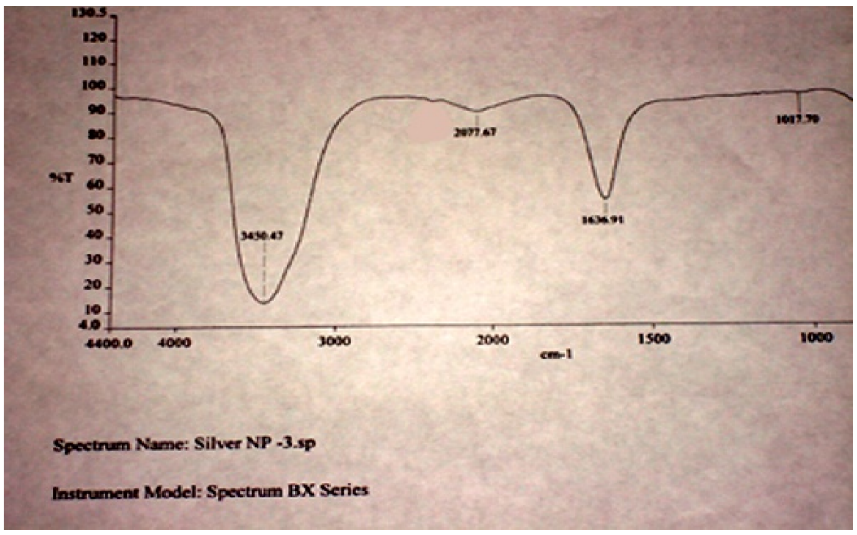

Figure 4. FTIR Spectroscopy Analysis of bioreduced silver nanoparticles

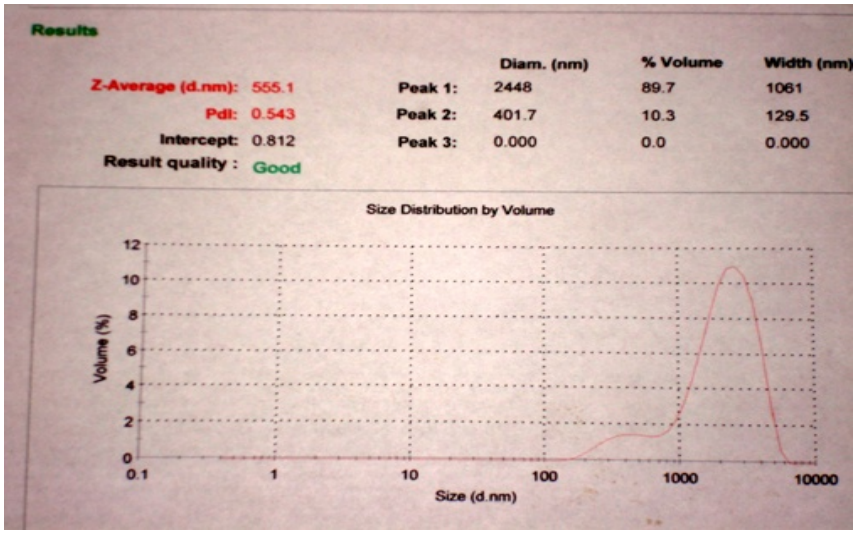

Figure 5. Particle size determination of bioreduced silver nanoparticles

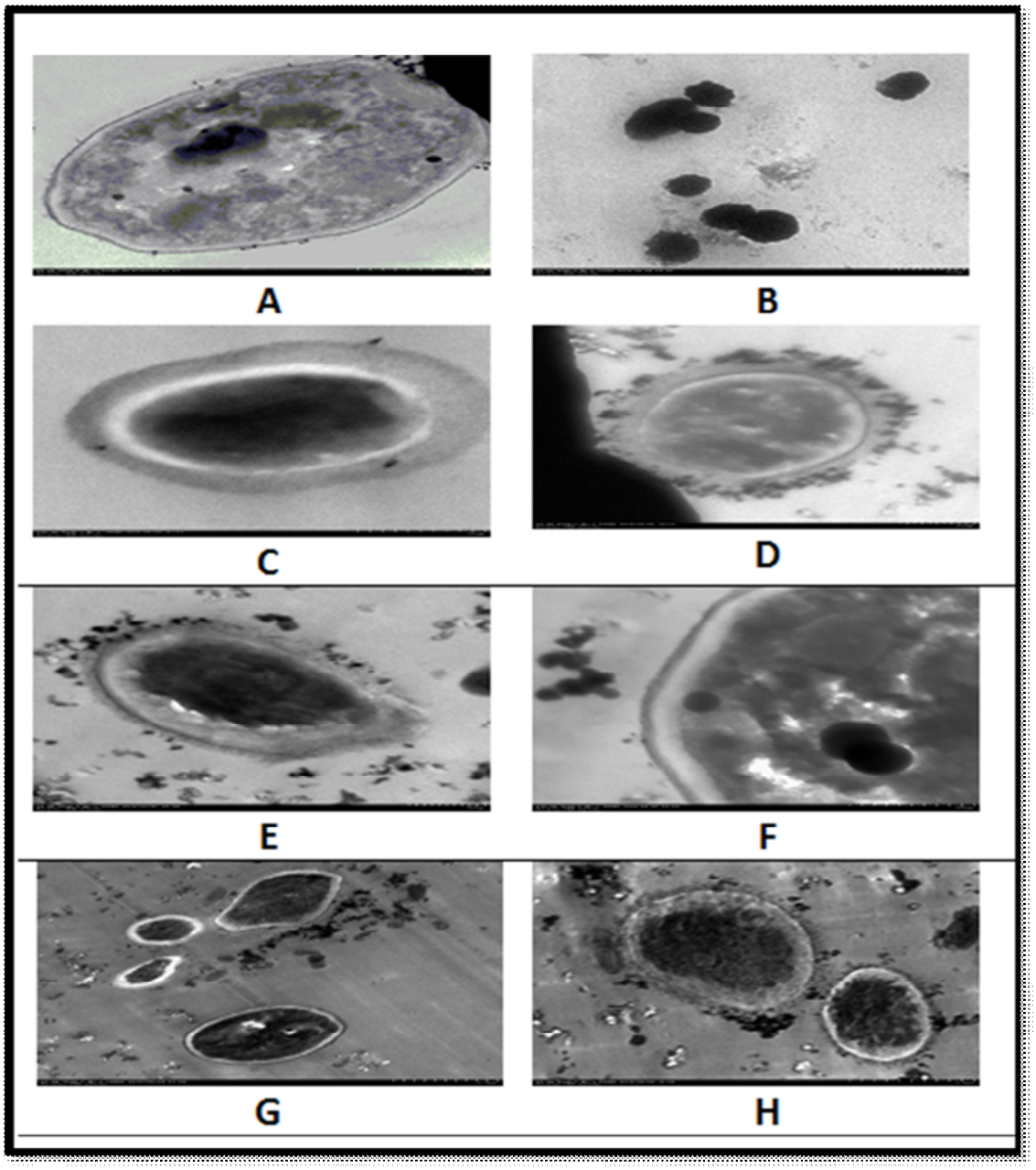

Figure 6. TEM images [low resolution] of *Actinotalea* reduced $AgNP_S$. A and F: Intracellular synthesis of SNP_S, B: SNPS were nearly spherical in shape, C, D and E: Extracellular synthesis of SNP_S, G and H: SNP_S formed between 2 bacterial cells