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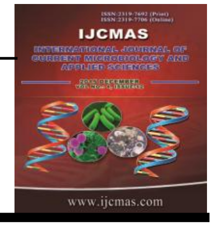

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**Original Research Article**

# Prodigiosin Mediated Biosynthesis of Silver Nanoparticles (AgNPs) and Evaluation of its Antibacterial Efficacy

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**A B S T R A C T**

Nanotechnology has recently emerged as an elementary division of science and technology that investigates and regulates the interaction of synthetic and biological materials. Nanotechnology is currently employed as a tool to explore the darkest avenues of medical sciences to combat diseases caused by drug resistant microbes. Silver nanoparticles (AgNPs) have been known for its inhibitory and bactericidal effects in the past decades. Silver Nanoparticles was synthesized by ecofriendly biogenic approach mediated by using the *Serratia marcescens* culture supernatant containing prodigiosin. The synthesized nanoparticles were characterized by UV-visible spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM), energy-dispersive spectroscopy (EDAX) and Transmission electron microscopy (TEM). AgNPs exerted maximum antibacterial activity against *E.coli* and *Pseudomonas* sp.

## Introduction

Nanotechnology has emerged as a rapidly growing research field intersecting with various other branches of science and technology for the purpose of manufacturing new materials at the nanoscale level. The word “nano” is used to indicate one billionth of a meter or $10^{-9}$ (Priya *et al.*, 2013). Nanoparticles are clusters of atoms in the size range of 1–100 nm. “Nano” is a Greek word synonymous to dwarf meaning extremely small. The term Nanotechnology was coined by Professor Norio Taniguchi of Tokyo Science University in the year 1974. Nanosized inorganic particles, of either simple or composite nature, display unique physical and chemical properties and represent an increasingly important material expressing significant advances owing to wide range of applications in the field of bio-medical, sensors, antimicrobials, catalysts, electronics, optical fibers, environmental purification, pharmaceuticals, agricultural, bio-labeling and in other areas (Sondi and Salopek-Sondi, 2004; Kavitha *et al.*, 2013).

Nanoparticles can be broadly categorized into two major types namely, organic nanoparticles including carbon nanoparticles (fullerness), inorganic nanoparticles including magnetic nanoparticles, noble metal nanoparticles (like gold and silver) and semi-conductor nanoparticles (like titanium dioxide and zinc oxide). There is a growing interest in inorganic nanoparticles i.e. of noble metal nanoparticles like gold and silver as they provide superior material properties with functional versatility and stability (Vadlapudi and Kaladhar, 2014). Methods employed for the synthesis of nanoparticles are broadly classified under two processes such as "Top-down" process and "Bottom-up" process. Top-down approach: Bulk material is broken down into particles at nanoscale with various lithographic techniques e.g.: grinding, milling etc. Bottom-up approach: Atoms self-assemble to new nuclei which grow into a particle of nanoscale (Kavitha *et al*., 2013).

Microbial drug resistance has emerged as a global health concern, as microbes acquire resistance by changing their metabolic activities and genetic structure. Nanotechnology is expected to open some new aspects to fight and prevent diseases using atomic scale tailoring of materials (Afreen *et al*., 2011). Nanotechnology is currently employed as a tool to explore the darkest avenues of medical sciences to combat diseases caused by drug resistant microbes (Singh *et al*., 2014). Silver nanoparticles (AgNPs) have been known for its inhibitory and bactericidal effects in the past decades (Cho *et al*., 2005). Antibacterial activity of AgNPs can be applied in biomedical applications such as reduction of infections on the burn treatment, for the treatment of various infectious diseases, prevention of bacterial colonization on catheters and elimination of microorganisms on textile fabrics as well as disinfection in water treatment (Yuranova *et al*., 2003; Chou *et al*., 2005; Shameli *et al*., 2012; Singh *et al*., 2014). The present study focuses on the biosynthesis, characterization and antibacterial activity of silver nanoparticles (AgNPs) produced in the presence of prodigiosin.

## Materials and Method

### Chemicals Used

$AgNO_3$ (99.98%) used as a silver precursor was procured from Sigma Aldrich Chemicals, Bangalore, India. All the solutions were freshly prepared using double distilled water and kept in the dark to avoid any photochemical reactions. All the glasswares used in experimental procedures were cleaned in a fresh solution of $HNO_3$/HCl (3:1, v/v), washed thoroughly with double distilled water and dried before use.

### Bacterial Strain Used

The bacterial strain used in this study was isolated from fish pickle and fish sauce samples procured from the local markets. Based on the morphological, cultural, biochemical characteristics and 16 s rDNA sequencing, the red pigmented (prodigiosin) bacterial isolate was identified as *Serratia marcescens*. The pure cultures of *Serratia marcescens* were maintained on nutrient agar slants at 4°C.

### Purification of Prodigiosin

The red pigmented bacterial stain *Serratia marcescens* was grown in nutrient broth containing (yeast extract, sodium chloride and peptone) and incubated at 37°C for three days. Following three days of incubation, cells were harvested by centrifugation at 8000 rpm for 10 mins. Then the pellet was washed with sterile distilled water and centrifuged at 8000 rpm for 10 mins. 2 ml of methanol was added to the pellet and centrifuged at 8000 rpm for 10 mins. The supernatant was collected and stored as the source of prodigiosin for further studies.

**Prodigiosin Mediated Silver Nanoparticle Preparation**

Silver nanoparticles were synthesized by using the *Serratia marcescens* culture supernatant containing prodigiosin. Equal volume of culture supernatant and 0.1 $AgNO_3$ solution was mixed and incubated in a water bath at 60 °C until color change was observed (pink to black color).The solution containing silver nanoparticles were separated and concentrated by repeated washing and centrifugation at 10,000 rpm for 15 mins. The final suspension was dried and nanoparticle obtained was used for further experimental studies.

**Characterization Techniques**

The prepared Ag-NPs were characterized by using the ultraviolet–visible spectroscopy, X–ray diffraction (XRD), scanning and transmission electron microscopy (SEM & TEM). The Ag nanoparticles were characterized in a Shimadzu UV-VIS Spectrophotometer. The scanning range for the samples was 300-800 nm. The double distilled water used as a blank reference. The purified and dried pellet of synthesized Ag NPs was subjected to X-ray diffraction (XRD) analysis. The XRD patterns were recorded at a scan speed of 2° $min^{-1}$. Meanwhile, the structures of the produced Ag-NPs were examined using Shimadzu PXRD–6000, powder X–ray diffraction. The particle size and morphology of the Ag NPs were examined using Scanning electron microscopic observations. The size of the NPs was confirmed by using TEM analysis (Transmission electron microscopy – Hitachi H-7100) using an accelerating voltage of 120 kV and methanol as solvent

**Antibacterial Activity of Silver Nanoparticles**

The antibacterial effect of Ag-NPs was examined by disc diffusion method against *Staphylococcus* sp, *E.coli* and *Pseudomonas* sp. collected from lab stock. Muller Hinton agar was prepared and poured onto the sterile petriplates. After solidification, 2 wells were cut (for test and control) and each culture was swabbed individually on the respective plates. The synthesized AgNPs nanoparticles were diluted with distilled water (15µg/ml) and placed onto each wells and incubated for 24 hours. Following incubation the zone of inhibition against nanoparticle were observed and measured.

**Results and Discussion**

**Synthesis of AgNPs**

Green synthesis of silver nanoparticles was carried out by using the culture supernatant of *Serratia marcescens* containing prodigiosin. On mixing prodigiosin with silver nitrate solution (1mM), a change in the color from pale yellow to dark brown was observed. Similar results were also reported by many researchers (Krishnaraj *et al*., 2010; Jayaseelan *et al*., 2011; Singh *et al*., 2014). The brown color confirms the reduction of $Ag^{+}$ which indicates the formation of Ag nanoparticles.

**Characterization of Ag Nanoparticles**

**UV–VIS Spectral Analysis**

UV–Vis spectroscopy is one of the most significant tools to characterize the metal nanoparticles. The absorption behavior arises due to surface Plasmon resonance (SPR), which originates from coherent oscillations of electrons in the conduction band of nanoparticles induced by the electromagnetic field. The SPR phenomenon arises when nanoparticles are irradiated with visible light, because of the collective oscillations of the conduction electrons (Anandh *et al*., 2014). In our results peak specific for the synthesis of silver nanoparticles was obtained at 400-425 nm by UV Visible spectroscope in the form of a sharp peak (Fig 1). It is well known that colloidal silver nanoparticles exhibit absorption at the wavelength from 390 to 420 nm due to Mie scattering (Singh *et al*., 2014).

**X-ray Diffraction Analysis**

X-ray diffraction is a very important method to characterize the structure of crystalline material and used for the lattice parameters analysis of single crystals, or the phase, texture or even stress analysis of samples. The crystal structure of the AgNPs was analyzed by X-ray diffractometer. The formation of silver nanoparticles synthesized using the culture supernatant of *Serratia marcescens* containing prodigiosin was supported by X-ray diffraction measurements. X-ray diffractogram of the synthesized AgNPs showed distinct diffraction peaks at 27.42°, 31.75°, 45.50°, 53.91°, 58.49°, 60.20°, 75.33° and 76.61° indexed to the planes 110, 111, 211 and 220 (Fig 2).

**SEM and EDAX Analysis**

The SEM images of silver nanoparticles nanoparticles obtained with the culture supernatant of *Serratia marcescens* containing prodigiosin is shown in the Fig 3. The formation of silver nanoparticles as well as their morphological dimensions in the SEM study demonstrated that the average size was from 25.9 nm - 34.8 nm with interparticle distance, whereas the shapes were uniformed spherical and ellipsoidal. Analysis through EDAX confirmed the presence of strong silver signal along with other elements, which might have originated from the biomolecules that are bound to the surface of nanosilver particles (Fig 4). Similar results were reported with the silver nanoparticles synthesized with the leaf and stem extracts of *Paederia foetida* and *Tinospora cordifolia* respectively (Lavanya *et al*., 2013; Singh *et al*., 2014).

**Fig 1** UV–VIS Spectral Analysis of AgNPs Synthesized using Prodigiosin

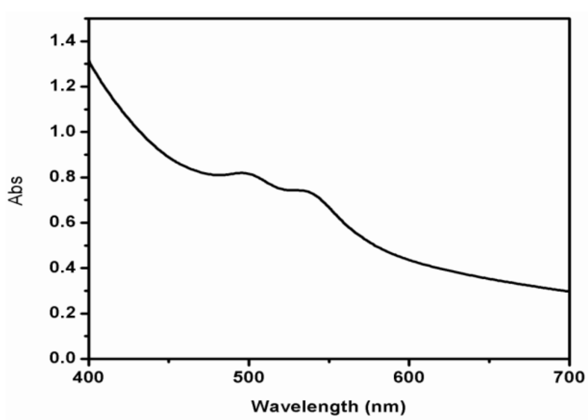


**Fig.2** XRD Analysis of AgNPs Synthesized using Prodigiosin

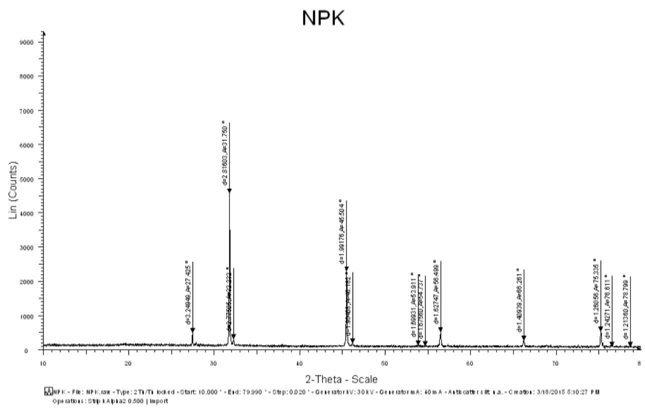



**Fig.3** SEM Images of AgNPs Synthesized using Prodigiosin

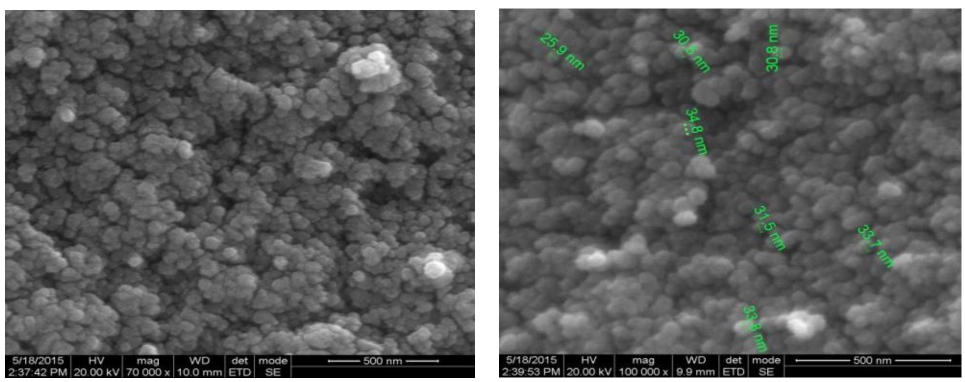



**Fig.4** EDAX Analysis of AgNPs Synthesized using Prodigiosin

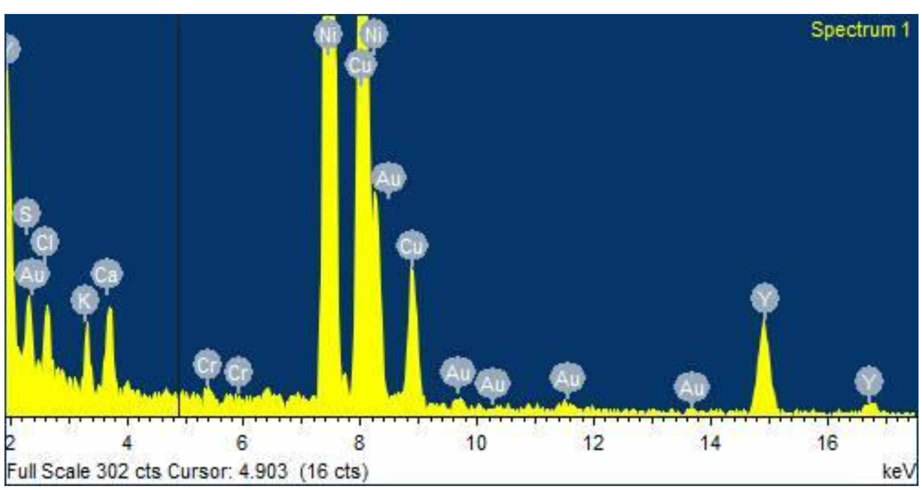
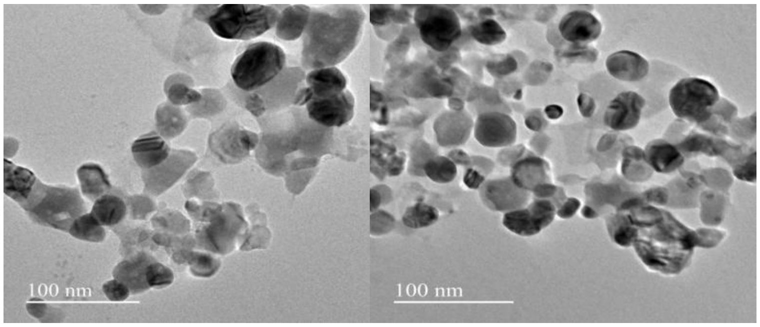


**Fig.5** TEM Images of AgNPs Synthesized using Prodigiosin




**Transmission Electron Microscopy**

The TEM images and their size distributions of silver nanoparticles obtained with the culture supernatant of *Serratia marcescens* containing prodigiosin is shown in Fig 5. From the images it is evident that the morphology of AgNPs are nearly spherical and some non-spherical in nature having particle size less than 100 nm. Silver nanoparticles synthesized with the stem extract of *Tinospora cordifolia* was reported to be 36 ± 9 nm (Singh *et al*., 2014). It is known that spherical as well as non-spherical (triangle or hexagonal) nanoparticles exhibits better physical properties, if they are produced in small size, as the antibacterial properties of silver nanoparticles are size dependent.

**Antibacterial Activity of Silver Nanoparticles**

AgNPs synthesized using prodigiosin exerted maximum antibacterial activity against *E.coli* and *Pseudomonas* sp. Silver ions have long been known to exert strong inhibitory and bactericidal effects as well as to possess a broad spectrum of antimicrobial activities (Berger *et al*., 1996). Silver ions cause the release of $K_+$ ions from bacteria; thus, the bacterial plasma or cytoplasmic membrane, which is associated with many important enzymes and DNA, is an important target site of silver ions (Kim *et al*., 2011). Because of their size, Ag-NPs can easily reach the nuclear content of bacteria and they present the large and impressive surface area; thus, the contacts with bacteria were the greatest. The antibacterial activity of silver nanoparticles synthesized by chemical means showed equal antibacterial activity against *S.typhimuium* (Gram -ve) and *S. aureus* (Gram +ve) (Shameli *et al*., 2012).

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