

Endophytes as Potential Nanofactories

Swetha Sunkar¹ and Valli Nachiyar²

Abstract—Nanobiotechnology has emerged in the recent past for developing facile, green and ecofriendly technology for synthesis of nanoparticles of variable size, shapes, chemical composition and controlled dispersity owing to their potential use for human benefits. The present study focuses on the biosynthesis of silver nanoparticles using one faction of microbes, the endophytes fungi as a “green” alternative to the chemical method. Some of the endophytes were able to synthesise silver nanoparticles that were characterised by UV-VIS spectroscopy, Transmission electron microscopy and FTIR analysis. It has been observed that the size of the nanoparticles to be in the range of 25-80 nm with a smooth surface. The nanoparticles displayed significant antimicrobial activity against certain standard pathogens. Endophytes have made an entry in the catalogue of benign synthesizers of bionanoparticles generating silver nanoparticles as the new age antimicrobials.

Keywords—Endophytes; silver nanoparticles; UV-vis spectra; SEM.

I. INTRODUCTION

IN the recent past paramount importance is given to research in the field of nanotechnology owing to its enormous applications in various fields. Metals in nanosize exhibit pronounced properties due to the increase in the surface to volume ratio and thereby altering the mechanical, thermal and catalytic properties of the material. The inorganic nanoparticles especially silver and gold are gaining importance as potential tools for medical imaging and treating diseases. Traditionally, nanoparticles are synthesized by physical and chemical methods but they pose certain problems in terms of the chemicals involved and harsh conditions employed. The focus on the biogenic routes to synthesis of nanoparticles paved the way for the search of new biosources capable to reduce metals to their nanosizes. In this line, plants and microorganisms like bacteria and fungi have been experimented and proved successful. But very few reports are available on the use of endophytic microorganisms for the synthesis of nanoparticles.

The term “endophytes” includes a suite of microorganisms that grow intra-and/or intercellularly in the tissues of higher plants without causing over symptoms on the plants in which they live, and have proven to be rich sources of bioactive natural products [1, 2]. The endophytes may provide protection and survival conditions to their host plant by

producing a plethora of substances which, once isolated and characterized, may also have potential for use in industry, agriculture, and medicine [3].

Approximately 300000 plant species growing in unexplored area on the earth are host to one or more endophytes [4], and the presence of biodiverse endophytes in huge number plays an important role on ecosystems with greatest biodiversity. Endophytes provide a broad variety of bioactive secondary metabolites with unique structure, including alkaloids, benzopyranones, flavonoids, phenolic acids, quinones, steroids, terpenoids, tetralones, xanthenes, and others [2]. Such bioactive metabolites find wide-ranging application as agrochemicals, antibiotics, immunosuppressants, antiparasitics, antioxidants, and anticancer agents [3]. The discovery of novel antimicrobial metabolites from endophytes is an important alternative to overcome the increasing levels of drug resistance by plant and human pathogens, the insufficient number of effective antibiotics against diverse bacterial species, and few new antimicrobial agents in development process. Hence an attempt has been made in this study to synthesize metal nanoparticles by employing endophytes isolated from various plants thus making the entry of endophytes into the register of nano-synthesizers is novel and interesting.

II. MATERIALS AND METHODS

A. Isolation of endophytes

Leaf samples were cleaned under running tap water to remove debris and then air dried and processed within 5 hrs of collection. From each leaf sample, 4 segments of 1 cm length were separated and treated as replicates. Surface sterilization was carried out by submerging them in 75% ethanol for 2 mins. The explants were further sterilized sequentially in 5.3% sodium hypochlorite (NaOCl) solution for 5 min and 75% ethanol for 0.5 min [5]. Samples were allowed to dry on paper towel in a laminar air flow chamber. Four segments per plant were placed horizontally on separate Petri dishes containing Potato Dextrose Agar (PDA) and nutrient agar (NA) for the growth of fungi and bacteria respectively. After incubation the endophytic fungi and bacteria were collected and placed onto PDA and NA and incubated for culture purity. Eventually, pure cultures were transferred to PDA and NA slants and subcultured regularly.

B. Culture conditions and synthesis of Silver Nanoparticles

The endophytic fungi obtained were grown aerobically in liquid broth containing malt extract powder, glucose, yeast extract, peptone. The culture flasks were incubated on room temperature at 27°C. The biomass was harvested after 7 days

Swetha Sunkar is an Assistant Professor at Department of Biotechnology, Sathyabama University, Chennai, India. (phone:+919940483184; fax: ; e-mail:swethauk78@gmail.com).

Valli Nachiyar is the Head of the department of Biotechnology, Sathyabama University, Chennai, India. (e-mail:vnachiyar@gmail.com).

of growth by sieving through a plastic sieve followed by extensive washing with sterile double-distilled water to remove any medium components from the biomass. Typically 20 g of biomass (wet weight) were brought into contact with 100 ml sterile double-distilled water for 72 hours at 27°C in an Erlenmeyer flask and agitated at 150 rpm. After incubation the cell filtrate was obtained by filtering using Whatman filter paper No 1. 100 mL of cell filtrate is challenged 1 mM silver nitrate and incubated under dark conditions.

The endophytic bacterial culture was grown in 100 mL of Luria Broth medium and incubated for 36 hrs at 37°C with shaking at 150 rpm. After incubation period, the bacterial cell pellet was collected by centrifugation at 10,000 rpm for 10 min. The pellet as well as the culture supernatant was challenged with 1mM silver nitrate solution and incubated under dark conditions [6].

C. Characterization of silver nanoparticles

The formation of silver nanoparticles (AgNPs) was followed by visual observation of color that changes from pale white to brown and was further confirmed by the sharp peaks given by the AgNPs in the visible region from UV – vis spectrum of the reacting solution using Perkin-Elmer Lamda-45 spectrophotometer, in a 1cm path quartz cell at a resolution of 1 nm from 250 to 800 nm. The studies on morphology, size and the distribution of nanoparticles were performed by Transmission Electron Microscopic (TEM) analysis using a TEM, JEM- 1200EX, JEOL Ltd., Japan, Scanning Electron Microscope (SEM) using Hitachi S-4500 SEM. The probable biomolecules involved in the synthesis and stabilization of nanoparticles was recorded by FTIR spectrum using FTIR Nicolet Avatar 660 (Nicolet, USA) [6].

III. RESULTS AND DISCUSSION

The biosynthesis of nanoparticles by microbes is thought to be clean, nontoxic, and environmentally acceptable “green chemistry” procedures. The rate of formation and size of the nanoparticles could, to an extent, be manipulated by controlling parameters such as pH, temperature, substrate concentration, and exposure time to substrate. Endophytes that are diverse in nature play a role in the hosts tolerance to stress, production of phytohormones, resistance against pathogens and herbivores, protection from insects, worm, pests and herbivores, enzymes production and houses a great variety of secondary metabolites like anticancer agents, antimicrobial agents, antioxidant agents and antiviral agents.

While a detailed study is being carried out on the medically and industrially important compounds from endophytes, not much focus is being put on the ability of endophytes to reduce metals to nanosizes. Hence this study aims to delve into this arena where endophytes are used for the synthesis of silver nanoparticles.

The endophytes were isolated from various plants in our earlier study [7] and they were used to screen for their ability to synthesize silver nanoparticles. There were a few interesting observations in this study. Firstly, not all the endophytic fungi and bacteria had the potential to synthesize AgNPs. Secondly,

extracellular synthesis using the culture supernatant was successful with fungi while the AgNP synthesis was mediated by the biomass of the bacteria.

The fungi that gave a positive result were ARA isolated from *Aravae lanata* and GX2, GX3 which were isolated from *Garcinia xanthochymus*. Two endophytic bacteria were showing positive results and were designated as ADA from *Justicia beddomei* and B-GX1 from *Garcinia xanthochymus*.

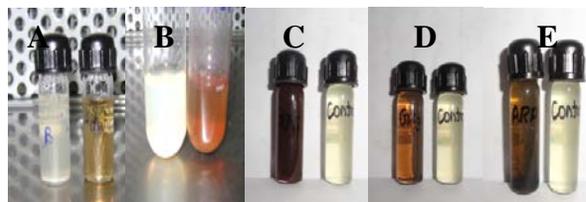


Fig 1. Colour change observed from pale white to brown. Bacteria A) ADA B) B-GX1 Fungi C) GX2 D) GX3 E) ARA

The formation of nanoparticles was observed from the colour change of the treated solution from pale white to brown that indicated the reduction reaction. (Fig 1) [8,9]. The characteristic brown color arises due to excitation of surface plasmon vibrations in the silver metal nanoparticles. The color intensity of the cell filtrate with AgNO₃ was sustained even after 24 h incubation, which indicated that the particles were well dispersed in the solution, and there was no obvious aggregation [10].

A. Characterization of AgNPs

This bioreduction of Silver nitrate ions was followed by UV-vis spectroscopy. The spectrums of the AgNPs showed a strong surface plasmon absorption band at around 400 nm (Table I).

Endophyte	Isolate	UV-Vis Peak (nm)
Fungi	ARA	423
	GX2	400
	GX3	435
Bacteria	ADA	433
	GX2	432

The UV-Vis spectra of the silver nanoparticles is shown in figure 2 that display clear peaks at around 400 nm that is characteristic of silver. The broad peaks indicate the presence of spherical or roughly spherical AgNPs that remained the same throughout the reaction period, suggesting that the particles are dispersed in the aqueous solution with no evidence for aggregation [11]. Observation of this sharp clear peak, assigned to a surface plasmon, was well documented for various metal nanoparticles with sizes ranging from 2 to 100 nm [12, 13].

Figure 2 shows the UV-vis spectra of the silver nanoparticles

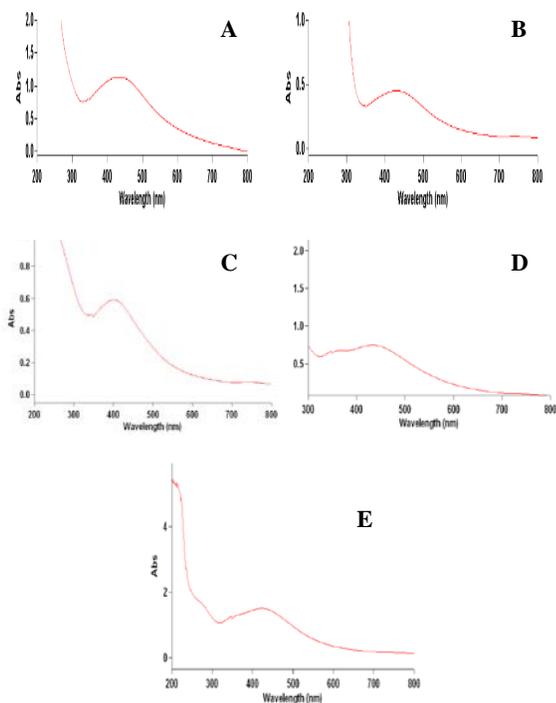


Fig 2. UV-Vis spectra of the AgNPs
Bacteria A) ADA B) B-GX1 Fungi C) GX2 D) GX3 E) ARA

B. Microscopic identification of AgNPs

The size and shape of the nanoparticles that plays a significant role in their function is identified by SEM and TEM analysis (Fig.4(a)). The SEM micrographs recorded showed comparatively spherical nanoparticles that were observed to be uniformly distributed. Their size was in the range of 25 – 80 nm [14].

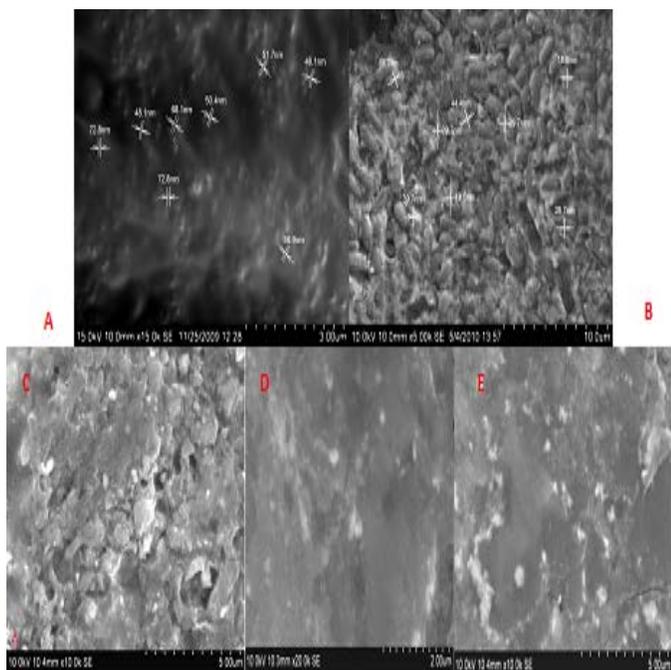


Fig 4(a). SEM Micrographs of the silver nanoparticles
Bacteria A) ADA B) B-GX1 Fungi C) GX2 D) GX3 E) ARA

The possible mechanism of the formation of silver nanoparticles by the culture supernatant is still not very clear. But the nature of the possible biomolecules involved in the synthesis can be identified by their FTIR spectra. The FTIR spectra of the bio-nanosilver is shown in figure 4(b).

Similar peaks were given by all the AgNPs. The broad peaks at 3447, 3448 and 3435 cm^{-1} corresponds to the N-H stretching vibrations amines. The two peaks at 2367/2364 cm^{-1} and 2344 cm^{-1} obtained in the spectra corresponds to the stretching vibrations of carboxylic acids. A small peak at 2068 and 2081/2081 cm^{-1} in the AgNPs is indicative of transitional carbonyls. The bands at 1638/1639 cm^{-1} correspond to the stretch molecule vibration while the peak at 722/730/715 and 701 cm^{-1} can be assigned to the aromatic C-H out of plane bending vibrations. This FTIR spectrum supports the presence of proteins in the synthesis of silver nanoparticles. [14].

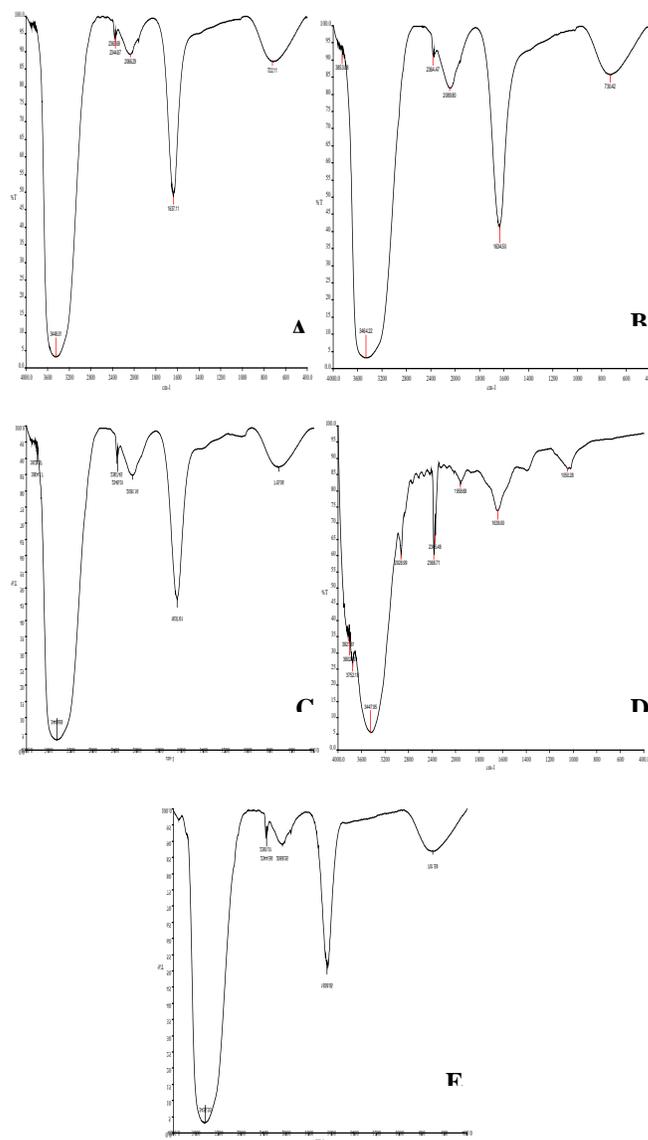


Fig 4 (b). FTIR spectra of the AgNPs
Bacteria A) ADA B) B-GX1 Fungi C) GX2 D) GX3 E) ARA

IV. CONCLUSION

The natural condition of plants seems to be in a close interaction with endophytes. Endophytes seem promising to increase crop yields, remove contaminants, inhibit pathogens, and produce fixed nitrogen or novel substances. The need to search new ecological niches for potential sources of natural bioactive agents for different pharmaceutical, agriculture, and industrial applications led to explore their potential to reduce metals adopting renewable, eco-friendly and easily obtainable methodologies.

ACKNOWLEDGMENT

The authors would like to thank Sathyabama University for their support in carrying out this research.

REFERENCES

- [1] J. Li, G.Z. Zhao, H.H. Chen, H.B. Wang, S. Qin S, W.Y. Zhu, L.H. Xu, C. L. Jiang, W.J. Li, "Antitumour and Antimicrobial Activities of Endophytic Streptomycetes from Pharmaceutical Plants in Rainforest", *Lett. Appl. Microbiol.*, vol 47, pp 574-580, 2008.
- [2] R.X. Tan, W.X. Zou, "Endophytes: a Rich Source of Functional Metabolites", *Nat. Prod. Rep.*, vol 18, pp 448-459, 2001.
- [3] G.A. Strobel, "Endophytes as Sources of Bioactive Products", *Microb. Infect.*, vol 5, pp 535-544, 2003.
- [4] G. Strobel, B. Daisy, "Bioprospecting for Microbial Endophytes and Their Natural Products". *Microbiol. Mol. Biol. Rev.*, vol 67, pp 491-502, 2003.
- [5] N. S. Ravi Raja, G. L. Maria, K. R. Sridhar, "Antimicrobial evaluation of endophytic fungi inhabiting medicinal plants of the Western Ghats of India". *Eng. Lifescience*, vol 6, pp 515-520, 2006.
- [6] Swetha Sunkar, C. Valli Nachiyar. "Microbial Synthesis and characterization of silver nanoparticles using the Endophytic bacterium *Bacillus cereus*: A novel source in the benign synthesis". *Global J Medical Research* vol 12, pp 43-49, 2012.
- [7] Swetha Sunkar and Valli Nachiyar "Endophytes: A Prospective Source of Enzyme Production" volume 6 no. 2 pp 859-867, 2012
- [8] S. Minaeian, A. R. Shahverdi, A. S. Nohi, H. R. Shahverdi." Extracellular biosynthesis of silver nanoparticles by some bacteria". *J. Sci. I. A. U (JSIAU)* vol 17, pp 1-4, 2008.
- [9] Z. Sadowski, I.H. Maliszewska, B. Grochowalska, I. Polowczyk, T. Ożlecki, "Synthesis of silver nanoparticles using microorganisms" *Mat Sci-Poland* vol 26, pp 419-424, 2008.
- [10] Swetha Sunkar and C. Valli Nachiyar, "Biogenesis of antibacterial silver nanoparticles using the endophytic bacterium *Bacillus cereus* isolated from *Garcinia xanthochymus*" *Asian Pac J Trop Biomed* vol 2, no12, pp 953-959, 2012.
- [11] N. Saifuddin, C. W. Wong, A. A. N. Yasumira "Rapid Biosynthesis of Silver Nanoparticles Using Culture Supernatant of Bacteria with Microwave Irradiation" *E-Journal of Chemistry* vol 6 no 1, pp 61-70, 2009.
- [12] M. Kowshik, S.H. Ashtaputre, S.H. Kharazi, "Extracellular synthesis of silver nanoparticles by a silver-tolerant yeast strain MKY3" *Nanotechnology* vol 14, pp 95-100, 2003.
- [13] A. Henglein "Physicochemical properties of small metal particles in solution: microelectrode reactions, chemisorption, composite metal particles, and the atom-to-metal transition". *J. Phys. Chem* vol 97, pp 5457-5464, 1993.
- [14] K. C. Bhainsa, S.F. D'Souza. "Extracellular biosynthesis of silver nanoparticles using the fungus *Aspergillus fumigates*" *Colloids and surfaces B Biointerfaces* vol 47, pp 160-164, 2006.