BIOSYNTHESIS OF AU NANOPARTICLES USING THE ENDOPHYTIC FUNGI ISOLATED FROM *BAHUNIA VARIEGATA* L.

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***Abstract*— The development of reliable and eco friendly process for the synthesis of nano scale materials is an important aspect in the field of nanotechnology. Colloidal chemical synthesis methods have produced an impressive range of homogeneously size-distributed forms. The chemical methods are complex and often variable from experiment to experiment, and the resulting crystals are often poorly compatible with water and/or biological cells. The biosynthesis of nano scale materials using the microorganisms and plant materials have emerged as an alternative for the chemical synthesis. The biosynthesis is more advantageous than the chemical synthesis due to the highly controlled reproducible synthesis, generation of water-soluble and biocompatible particles. Since the production doesn't involve any chemicals such as toxic surfactants or organic solvents, the process is 100% eco friendly, low cost and nanoparticles toxicity is reduced. In the present investigation, the endophytic fungi isolated from the medicinal plant B*auhinia variegata* Linn. mediate for the reduction of gold substrate, Chloroauric acid of Au^{3+} to Au^{0}. The reduction was indicated by the color change of the substrate and also by using the UV Spectrophotometric analysis at a wavelength range of 200 nm to 700nm. The synthesized nanoparticles were harvested by centrifugation at 13000 rpm for a period of 15 minutes.**

Keywords-*, *endophytic fungi, Bahunia Variegata* L. *Choloauric acid

1. INTRODUCTION

Nanotechnology has dynamically developed as an important field of modern research with potential effects in electronic and medicine(1,2,3). The term nanotechnology was coined by Norio Taniguchi, in 1974 to describe the processing of separation, consolidation and deformation of materials by one atom or one molecule. Although the science of nanotechnology is only four decades old, it has grown fast and had diversified, encompassing new approaches based upon molecular self assembly to for developing new materials of less than100nm range. Nanotechnology can be defined as a research for the design, synthesis, and manipulation of structure of particles with dimension smaller than 100nm.

A new branch of nanotechnology is nanobiotechnology. Nanobiotechnology combines biological principles with physical and chemical procedures to generate nano-sized particles with specific functions. Nanobiotechnology represents an economic alternative for chemical and physical methods of nanopaticles formation. These methods of synthesis can be divided on intra cellular and extracellular(4). The biomimetic and biomineralization procedures for nanoscale material synthesis are expected to yield novel and complex structural entities compared with those obtained by the conventional methods.

In recent years the synthesis of gold nanoparticles have been the focus of intense interest because of their emerging applications in a number of areas such as bioimaging, biosensors, biolabels, biomedicines, and so forth(5,6,7,8). Conventionally gold nanoparticles are usually synthesized by reducing a gold salt ($NaAuCl_4$) with sodium citrate ($Na_2H(C_3H_5O(COO)_3)$) or Sodium borohydride ($NaBH_4$) followed by surface modification with suitable capping ligands, occasionally organic solvents, which often raise environmental questions(9,10,11). However, these methods are complex and often variable from experiment to experiment, and the resulting crystals are often poorly compatible with water and/or biological cells.

The use of microbial cells for the synthesis of nano sized materials has recently emerged as a novel approach for the synthesis of metal nanoparticles[(1)]. Advantages of these methods include tightly controlled, highly reproducible syntheses; the generation of water-soluble, biocompatible particles; and the avoidance of toxic surfactants or organic solvents, which are compactable with water and/or biological cells. The biosynthetic method employing plant extracts has received some attention as a simple and viable alternative to chemical procedures and physical methods synthesizing metal nanoparticles only in recent years.

In the present investigation, the endophytic fungi isolated from *Bauhinia variegata* Linn. (Caesalpiniaceae), having Kachanar as the local Indian name, found in the Bannari Amman Institute of Technology, Sathyamangalam Campus, Tamil Nadu were used to synthesis the Gold nanoparticles by reducing the Chloroauric acid ($HAuCl_4$) substrate purchased from Sigma Aldrich and the analysis were carried out using UV-Visible spectrometric analysis.

2. MATERIALS AND EXPERIMENTAL DETAILS

Isolation and identification of endophytes

The plant parts leaves, flower, bud and tender stem of the medicinal plant *Bauhinia variegata* Linn. Wre collected from the Herbal Graden of Bannari Amman Institute of Technology, Sathyamangalam campus, were chopped into 3mm size parts and were surface sterilized using 1% $HgCl_2$. The surface sterilized plant parts were placed on Potato Dextrose Agar (PDA) plates with Amphicilin under sterile conditions and the plates were maintained at room temperature for the fungal development of fuga popagules.The developed colonies were purified by subculturing on Potato Dextrose Agar plates. The sporulating isolates were identified down to species level with the help of standard manuals [(13)]. Sterile isolates that cannot be assigned to any taxonomic group were sorted into sterile morphospecies on the basis of colony surface texture, hyphal pigmentation, exudates, and growth rates as described by Frolich et. al.,[(14)]. Such sterile forms are included as species for the analysis of results and the pure cultures for future use in Potato Dextrose Agar slants were stored at 20^0C.

Extracellular synthesis of Au nanoparticles

The fungal isolates were transferred to liquid media by inoculating a 1 cm diameter mycelium from a 7 day old PDA Petri dish into a 250 ml Erlenmeyer flask containing 100 ml liquid MGYP medium (0.3% w/v malt extract, 1.0% w/v glucose, 0.3% w/v, yeast extract and 0.5% w/v peptone) sterilized at 120^0C for 15 minutes. The fungi were then allowed to form a fungal mat on MGYP medium for a period of 14days at room temperature.The fungal mycelia were harvested from the media by filtration using man filter paper No.1. The filtrate was washed twice using deionized water and resuspended in 100 ml of deionised water and kept for 24 hours shaking at 30^0C. After incubation, the filtrate was obtained by filtration using Whatman filter paper No.1 and to synthesize gold nanoparticles, $HAuCl_4$ solution was added to the filtrate to make the final concentration to 1 mM and was kept for shaking.

UV spectrophotometric analysis

The reduction of Chloroauric acid was monitored visually as well as by UV-visible spectroscopic measurement. UV-visible spectrophotometric measurement of the reduced solution was recorded on a Perkin Elmer Lambda 35 UV visible spectrophotometer from a wavelength range of 200 to 700nm.

Harvesting of nanoparticles

The reduced filtrate containing gold nanoparticles were initially centrifuged at 2000rpm for 5 minutes to remove the micro particles present, if any. The supernatant was transferred to fresh centrifuge tube and centrifuged at 13000 rpm for 15 minutes for separating the nanoparticles.

3. RESULTS AND DISCUSIION

The isolated colonies subcultured on PDA plates were identified by the Lacto Phenol cotton blue staining. Using the morphological analysis through microscope and standard manual the following fungi were identified as: *Asperigillus niger, Penicillium citrinum., Colletotrichum gloeosporioides*, *Collectotrichum lindemuthianum* , *Collectotrichum circinallis*, *Phyllosticta* Sp. and *Phoma* Sp. Out of these isolates *Penicillium citrinum*, *Colletotrichum gloeosporioides, Collectotrichum lindemuthianum* and *Phyllosticta* Sp. (Fig. 1) were screened for their capability of synthesizing Au nanoparticles.

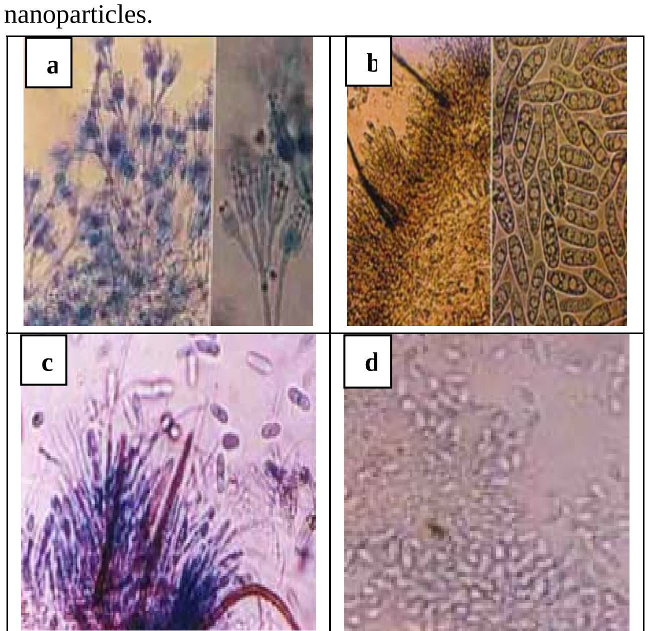

Figure 1. Organisms screened for nanopareticle synthesis a) *Penicillium citrinum,* b) *Colletotrichum gloeosporioides* c) *Collectotrichum lindemuthianum,* d) *Phyllosticta Sp.*

The choloroauric acid containing Au^{3+} was reduced to Au^{0} ionic form by the metabolites and enzymes present in the filtrate. In microbial synthesis, the reduction of Au^{3+} to ionic form was indicated by the color change of the filtrate from slight yellow to purple followed by the addition of chloroauric acid. Out of the screened endophytes only *Pencillium citrinum* showed the positive result. On comparison with other reported works of reduction happening in few minutes, the synthesis required more than 1 day for the color change to happen(15,16,17). The gold nanoparticles show a characteristic absorbance range of 510 nm to 560 nm (18). The UV Spectrophotometric analysis scanning of the extracellular gold nanoparticles synthesis by *Penicillium citrinum* at a wavelength range of 200 nm to 700 nm, showed a maximum absorbance peak at 544 nm, which is coming under the characteristic absorbance peak (Figure 2). The nanoparticles synthesized were separated by centrifugation. The harvested nanoparticles showed a golden brown color appearance.

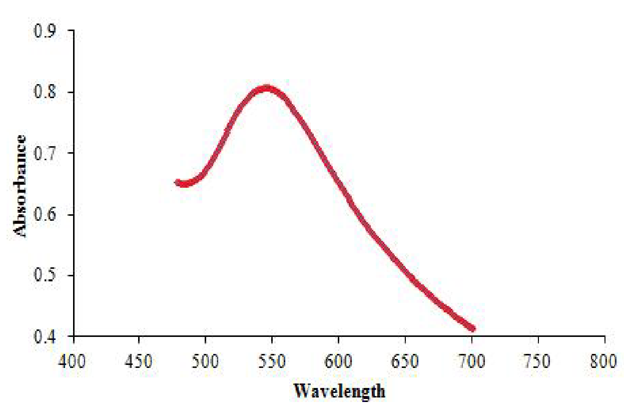

Figure 2. UV Spectrophotometric analysis of Au syntheis using *Penicillium citrinum*

4. CONCLUSION

In summary, biological synthesis of gold nanoparticles using the various endophytic fungi, recorded from *Bauhinia variegate* and the leaf and flower extracts of *Bauhinia variegate*.L has been demonstrated and it was concluded that the biosynthesis is more advantageous than the chemical synthesis due to the highly controlled reproducible synthesis, generation of water-soluble and biocompatible particles. Since the production doesn't involve any chemicals such as toxic surfactants or organic solvents, the process is 100% eco friendly and the nanoparticles toxicity is eliminated. Furthermore the low cost of the method as well as its simplicity and efficiency offers an alternative to chemical synthetic methods of gold nanoparticles. Achievement of such a green synthesis of gold nanoparticles, contributes to a raise in the efficiency of synthetic procedures using environmentally benign natural resources.

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