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Short Communication

Biosynthesis of gold nanoparticles by *Pseudomonas veronii* AS41G inhabiting *Annona squamosa* L.

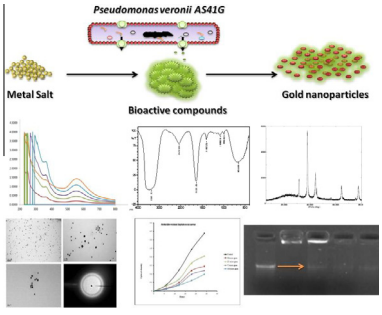
Syed Baker [a], Sreedharamurthy Satish [a,b,*]

[a] Bionanotechnological Laboratory, Department of Studies in Microbiology, Manasagangotri, University of Mysore, Mysore-570006, India
[b] Department of Plant Pathology, University of Georgia, Athens 30602, USA

HIGHLIGHTS

- Rapid synthesis of gold nanoparticles using *Pseudomonas veronii* AS41G.
- First report on the synthesis of gold nanoparticles from *Pseudomonas veronii* AS41G.
- Antibacterial activity of gold nanoparticles.
- Mode of action of gold nanoparticles on DNA.

GRAPHICAL ABSTRACT



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ABSTRACT

Biogenic principles to nanotechnology have generated tremendous attention in recent past owing eco friendly benign process for synthesis of nanoparticles. Present investigation reports extracellular synthesis of gold nanoparticles using cell free supernatant of *Pseudomonas veronii* AS 41G, a novel endophyte isolated from *Annona squamosa* L. Gold nanoparticles formation was confirmed with UV–Visible spectrophotometer. FTIR analysis predicted various functional groups responsible for reduction of metal salts and stabilization of gold nanoparticles. Nanoparticles were crystalline in nature as shown in XRD pattern. TEM analysis revealed morphological characteristics of nanoparticles with different size. Thus the present study attributes for facile process for synthesis of gold nanoparticles as an alternative for conventional methods. The study also highlights the new role of novel bacterium *Pseudomonas veronii* AS41G which will be very valuable as a record for the researchers working on it.

Introduction

Interdisciplinary research and improved scientific knowledge have contributed to significant progress in the field of biology especially with the emergence of nanotechnology which has created huge impact on all spheres of human life. Especially with the integration of nanoparticles which are reported to have innumerable applications [1]. Use of gold has been traced down since ancient records but with the invention of gold nanoparticles resulted in expansion of its applications. Gold nanoparticles are reported to bear innumerable applications in therapeutic, catalysis, biosensing, drug delivery, cancer treatment, etc. [2]. One of the important aspect of nanotechnology deals with nanoparticles synthesis. Various conventional methods are used for the synthesis of nanoparticles but these methods are bound with limitations. Hence biological route have gained popularity in recent years [3]. Biological entities such as prokaryotes to eukaryotes including plants are actively exploited for synthesis of nanoparticles. However use of plant species may pose a risk and imbalance to

* Corresponding author at: Herbal Drug Technological Laboratory, Department of Studies in Microbiology, University of Mysore, India. Tel.: +91 8212419734.
E-mail addresses: syedbaker3@gmail.com (S. Baker), satish@uga.edu, satish.micro@gmail.com (S. Satish).

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plant diversity especially when it comes to endangered species. Hence use of microorganisms could be prominent and more advantageous over other biological species. Perusal of scientific literatures reports different microorganisms used for the synthesis of nanoparticle. Compared to other microorganisms endophytic plethora are reported to secrete unique structurally diverse bioactive compounds which are known to mediate the synthesis of nanoparticles. Interference of endophytes and nanomaterials is relatively new area giving rise to unequivocal attention. Endophytes are microorganisms colonizing living, healthy internal tissues of plants without causing any immediate, overt negative effects. These endophytes are reported to perform myriad biological activities which influence its host for survival [4]. Thus the present study reports rapid synthesis of gold nanoparticles using *Pseudomonas veronii* AS41G a novel bacterium inhabiting *Annona squamosa* L.

Experimental procedure

Isolation of endophytes

The host plant *A. squamosa* L. was surveyed and samples were randomly collected from its natural habitats in southern India including the campus of University of Mysore. Plant materials were collected with cut ends sealed with parafilms and collected in paper bags which were carried to laboratory. The samples were than washed thoroughly under running tap water for 5–8 min followed by wash with sterile distilled water to remove the adhered debris and immersed in double distilled water containing 50 μg/ml of cycloheximide for 60 min to suppress the growth of fungal endophytes. Stem and leaves were subjected to surface sterilization under aseptic condition by sequential steps of immersing in 3.15% sodium hypochlorite for 5 min and then followed by ethanol 70% for thirty seconds. After successive surface sterilization the stem and leaves tissues were rinsed three times in sterilized distilled water and aseptically cut into small pads (0.5×0.5 cm^2) and placed on nutrient agar supplemented with 250 μg/ml of cycloheximide and incubated till bacterial endophytic colonies are visible [5,6].

Extracellular synthesis of nanoparticles

P. veronii AS41G was cultured in nutrient broth and incubated for 72 h. Later the culture broth was centrifuged at 8000 rpm at 4 °C for 20 min and supernatant was challenged with 10^{-3} mM gold chloroaurate and incubated on rotary incubator at 25 °C with 180 rpm. Parameters such as pH, temperature and concentration were varied to achieve stable gold nanoparticles with less time duration. The reduction of metal salts and production of gold nanoparticles was monitored by drawing samples periodically and analyzed by UV–Visible spectroscopy by recording the spectra between 200 and 800 nm by sampling 1 ml at different time intervals using Shimadzu double beam spectrophotometer.

Characterization of gold nanoparticles

FTIR spectroscopy analysis conferred functional group of biomolecules responsible to mediate the synthesis on a JASCO FT-IR 4100 instrument at room temperature with a resolution of 4 cm^{-1}. For XRD studies nanoparticles were coated on XRD grid and spectra were recorded by Rigaku Miniflex-II Desktop X-ray diffractometer instrument operating at a voltage of 30 kV. Size and morphology of gold nanoparticles was evaluated by TEM, an aliquot of an aqueous suspension of gold nanoparticles was transferred on to a carbon-coated copper TEM grids. The films on the TEM grids were allowed to stand for 2 min, then extra solution was removed and the grid was allowed to dry prior to measurement and scanned using a TECHNAI-T_{12} JEOL JEM-2100 Transmission electron microscope operated at a voltage of 120 kV with Bioten objective lens. Subsequently, the particle size was ascertained using a Gatan CCD Camera [6].

Phenotypic and genotypic characterization

Phenotypic characterization of the strain was carried out based on the tests for Gram staining, Oxidase and Catalase. Fluorescence pigment production was tested on Kings B agar and temperature tolerance was tested by growth at 4 °C and 42 °C [7]. Total genomic DNA was extracted and DNA pellets were resuspended in distilled water and stored at 20 °C. Amplification of 16S rDNA gene was performed as described earlier using universal primers, fP1 (5′-AGTT TGATCCTGGCTCA-3′) and rP2 (5′-ACGGCTACCTTGTTACGACTT-3′) [8]. The PCR products were purified, sequenced and deposited at GenBank (accession number KC480604). Similarity search for sequences was carried out using the BLAST function of GenBank. The 16S rDNA sequences of the isolate were aligned with other related species 16S rDNA sequences obtained from GenBank to construct phylogenetic trees by unweighted pair group of arithmetic mean analysis method and topologies of tree were evaluated by bootstrap method using the Limrr software.

Antibacterial activity and mode of action on DNA

Antibacterial activity of gold nanoparticles was evaluated via broth dilution assay against *Escherichia coli* (MTCC 7410) and *Staphylococcus aureus* (MTCC 7443) with concentration of 10^6 CFU (colony forming unit) suspensions respectively. Test organisms were treated with different concentration of biosynthesized gold nanoparticles ranging from 25 to 100 μg ml^{-1} and incubated at 37 °C for 24 h. Samples were drawn periodically at regular intervals and optical density was measured at 600 nm for growth of bacteria. Mode of action of gold nanoparticles was determined on DNA extracted from *S. aureus* using alkaline lysis based on the protocol described by Sambrook and Russell. Purity of the DNA was measured at optical density 260/280. Biosynthesized gold nanoparticles were treated with 10 ng of DNA and incubated at 37 °C. Later the mixture was evaluated by 1% agarose gel electrophoresis [9].

Results and discussions

Isolation of endophyte

Sequential surface sterilization eliminated surface flora on plant materials which was confirmed with no growth of colonies in the control plate. In addition media supplemented with cycloheximide suppressed the growth of fungi. Bacterial endophyte mediating gold nanoparticles was characterized and BLAST analysis revealed the bacterium sharing similarity with *P. veronii*. The sequence was deposited at GenBank with accession number KC480604. Upon phylogenetic analysis displayed the affiliation of *P. veronii* AS41G with other groups of Fluorescent *Pseudomonas*, Fig. 1, which was constructed using Limrr software. Our previous finding reported the synthesis of silver nanoparticles from the same bacterium, whereas in the present investigation gold nanoparticles were synthesized [6]. Earlier report suggests that geranium leaves (*Pelargonium graveolens*) and its endophytic fungus mediated extra-cellular synthesis of gold nanoparticles. Study reports rapid reduction of the metal ions with stable gold nanoparticles of variable size [10]. Similar result was also observed in the present study with rapid reduction of gold nanoparticles with varied size and shape.