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ORIGINAL ARTICLE

Biogenic nanoparticles bearing antibacterial activity and their synergistic effect with broad spectrum antibiotics: Emerging strategy to combat drug resistant pathogens

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Abstract The present study emphasizes on synthesis of bimetallic silver–gold nanoparticles from cell free supernatant of *Pseudomonas veronii* strain AS41G inhabiting *Annona squamosa* L. The synthesized nanoparticles were characterized using hyphenated techniques with UV–Visible spectra ascertained absorbance peak between 400 and 800 nm. Possible interaction of biomolecules in mediating and stabilization of nanoparticles was depicted with Fourier transform infrared spectroscopy (FTIR). X-ray diffraction (XRD) displayed Bragg's peak conferring the 100, 111, 200, and 220 facets of the face centered cubic symmetry of nanoparticles suggesting that these nanoparticles were crystalline in nature. Size and shape of the nanoparticles were determined using Transmission electron microscopy (TEM) micrograph with size ranging from 5 to 50 nm forming myriad shapes. Antibacterial activity of nanoparticles against significant human pathogens was conferred with well diffusion assay and its synergistic effect with standard antibiotics revealed 87.5% fold increased activity with antibiotic "bacitracin" against bacitracin resistant strains *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumoniae* followed by kanamycin with 18.5%, gentamicin with 11.15%, streptomycin with 10%, erythromycin with 9.7% and chloramphenicol with 9.4%. Thus

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the study concludes with biogenic and ecofriendly route for synthesizing nanoparticles with antibacterial activity against drug resistant pathogens and attributes growing interest on endophytes as an emerging source for synthesis of nanoparticles.

1. Introduction

Nanoparticles have burgeoning interest in recent years owing to their innumerable applications in interdisciplinary area of science. Noble metal nanoparticles are widely used in biosensing, biocatalysts, drug delivery, semiconductor, fuel cells, fluorescent probe, and antimicrobial agents (Baker et al., 2013). One of the important aspects of nanoparticles relies on synthesis process. Nanoparticles can be synthesized via physical and chemical methods and these processes are bound with various limitations such as use of toxic elements, generation of environmental pollutant and high energy which restricts the use of nanoparticles in biomedical applications (Li et al., 2011). Hence as an alternative approach biogenic principles are employed for safe and ecofriendly processes to synthesize nanoparticles which involve use of biological entities. In recent years nanoparticles are synthesized by using prokaryotic bacteria and eukaryotic organisms such as fungi and plants or their products (Baker et al., 2013).

However use of plant species may pose a risk and imbalance to plant diversity especially when it comes to endangered species; hence, microorganisms have better advantage over plants as they are inexhaustible resource. Microorganisms inhabiting unique biological niches are of great interest as they are capable of secreting diverse bioactive compounds bearing biological activities, and one such clique of microorganisms are endophytes (Baker and Satish, 2012b). Endophytes are microorganisms colonizing 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 during extreme conditions. Research on endophytes has yielded potential drug lead compounds with antibacterial, antiviral, antioxidant, insulin mimetic, anti-neurodegenerative and immunosuppressant properties (Strobel, 2003).

But interface between endophytes and nanomaterials is a relatively new and unexplored area which may open avenues in future to push forward the frontiers in coming decades (Baker and Satish, 2012a). Exploiting endophytic flora in synthesis of nanoparticles can lead to significant advances due to the fact that they secrete secondary metabolites bearing structurally diverse chemical compounds capable of reducing the metal salts and stabilize the nanoparticles as reported in various literatures (Sunkar and Nachiyar, 2013). The present investigation offers a valuable contribution to antimicrobial resistance area with synthesized nanoparticles bearing antibacterial activity against the drug resistant bacteria. Biosynthesis of nanoparticles is a growing area in bionanotechnology to unleash the ecofriendly approach as an alternative over conventional methods. The results emphasizes emerging role of endophytes toward synthesizing stable nanoparticles but reports of endophytic bacteria for nanoparticles synthesis are few which prompted us to isolate bacterial endophyte capable of nanoparticle synthesis which resulted in isolation of novel endophyte *Pseudomonas veronii* AS41G from *Annona squamosa* L. and employed for the synthesis of bimetallic nanoparticles and their antibacterial activity against significant human pathogens with synergistic effect using standard antibiotics conjugated with nanoparticles. Bioconjugation offers tremendous interest in biology, and biomolecules can be tailored with various other molecules but in recent years conjugation of nanoparticles with biomolecules has significant impact. The choice of the bioconjugation procedure depends strictly on physicochemical and biochemical properties of nanomaterials and bioactive molecules. The interaction between nanoparticles and biomolecules based on the electrostatic forces forms an organization resulting in functionalized nanoparticles in a reversible manner based on the opposite charges. As nanoparticles posses unique properties which form a base toward developing hybridization with the biomolecules resulting in a specific activity (Bagwea et al., 2003). Therefore, such interactions are promising enough toward developing bioconjugated nanoparticles as antimicrobial agents.

2. Materials and method

2.1. Materials

All the chemicals employed in the present investigation were procured from reputed international firms such as M/s Hi media and Sigma Aldrich. Silver nitrate and Chloroauric acid were obtained from Sigma Aldrich. Nutrient media and other chemicals were obtained from Hi media.

2.2. Surface sterilization

P. veronii strain AS 41G was isolated from surface sterilized leaf segment of *Annona squamosa* L. Surface sterilization was carried out with sequential steps by immersing plant material in 3.15% of sodium hypochlorite for five minutes 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 ($0.5 \times 0.5\ cm^2$) and placed nutrient agar supplemented with 250 μg/ml of cycloheximide and incubated till bacterial endophytic colonies are visible (Baker et al., 2015).

2.3. Synthesis of bimetallic silver–gold nanoparticles

Pure colony of actively growing *P. veronii* AS 41G was inoculated into nutrient broth and incubated for 72 h. Later the culture broth was centrifuged at 8000 rpm at 4 °C for 20 min to obtain cell free supernatant. 90 mL of stock solution with 1:1 of 1 mM $AgNO_3$ and $HAuCl_4$ was prepared. 10 ml of cell free supernatant was added to 90 ml of stock solution and