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Short Communication

Endogenic mediated synthesis of gold nanoparticles bearing bactericidal activity

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ABSTRACT

The present investigation aimed to synthesize gold nanoparticles using *Pseudomonas fluorescens* 417 inhabiting *Coffea arabica* L. Biologically synthesized gold nanoparticles were polydispersed in nature and characterized using hyphenated techniques such as UV-visible spectrophotometry, which ascertained characteristic peaks between 450 nm and 650 nm. Fourier transform infrared analysis predicted the functional groups present in the cell-free supernatant that mediated the synthesis and stabilization of gold nanoparticles. The crystalline nature of the gold nanoparticles was analyzed with X-ray diffraction techniques that displayed the Bragg's diffraction intensity. Transmission electron microscopy revealed the size of nanoparticles ranging from 5 nm to 50 nm, with most of them bearing a spherical shape. The study also revealed the bactericidal activity of synthesized nanoparticles against a panel of clinically significant pathogens. Maximum activity was observed against *Pseudomonas aeroginosa* followed by *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Klebsiella pneumoniae*. The results obtained in the present investigation are promising for ecofriendly approaches for synthesis of gold nanoparticles bearing bactericidal activity that can act as an alternative to combat drug-resistant pathogens.

1. Introduction

Use of nanomaterials can be traced from ancient times, but in recent times, their application and importance has increased [1]. Availability of technical resources and advances in scientific domains has led to emergence of nanotechnology and application of nanomaterials [2]. These nanomaterials have superior properties compared to their bulk counterparts. In recent years, nanomaterials have become a subject of interest among the scientific community, with many applications being explored. However, strict regulations have resulted in a decline in the use of these nanomaterials in biomedical applications. Their synthesis protocols involve the use of toxic materials, generate a lot of heat, and often require sophisticated infrastructure, which are barriers for many studies [3]. In order to overcome the limitations posed by these conventional methods, there has been a growing demand to develop ecofriendly and rapid synthesis of nanomaterials with the desired size and shape. Consequently, researchers have developed biogenic principles to synthesize nanomaterials by using biological resources such as plants and microorganisms or their products [4]. The use of biological entities is linked to their phyto- and bioremediation activities, and their ability

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to act as nanofactories to synthesize nanoscaled structures [5].

Most biogenic nanomaterials synthesized using diverse microbial flora are metallic nanomaterials such as silver and gold nanoparticles. Use of silver in therapeutics is well documented but the introduction of silver nanoparticles has resulted in expansion of its applications [6]. Silver nanoparticles are used as semiconductors, nanodevices, cosmetics, and biosensors, and in recent years, silver nanoparticles have been considered as potent antimicrobial agents against drug-resistant microorganisms [7]. Similarly, gold has been used for centuries in curing various aliments, and recently gold nanoparticles have demonstrated significant advances in medicine such as drug delivery, biocatalysts, and biolabeling [8]. Among the biological entities, the use of microorganisms in the synthesis of nanoparticles has successfully competed with conventional methods in synthesizing size-dependent nanomaterials. Microorganisms can synthesize nanomaterials in aqueous solutions, and the nanomaterials can be easily separated, thus becoming ecofriendly and cost-effective [9]. Microorganisms are an inexhaustible resource that can be preserved and reused, unlike plants, which can only be used once [10]. This causes an imbalance to plant diversity, especially among endangered species, and gives microorganisms an advantage over their plant counterparts [10]. Among the microbial community, plant-associated symbionts, "endophytes", have made a significant impact by secreting diverse secondary metabolites with biological activities. Although there has been significant research on endophytes, interference of endophytes in synthesizing nanomaterials is at an early stage and can generate major advances [11]; hence, the present emphasis on isolation of endophytic bacteria inhabiting *Coffea arabica* L. in Southern India. Isolated endophyte was characterized to reveal its close affinity to *Pseudomonas fluorescens*. Upon treatment with the metal salt gold chloroaurate, the endophytes were capable of rapid extracellular synthesis of gold nanoparticles bearing bactericidal activity against a panel of test pathogenic microorganisms. Here, we report an ecofriendly approach for synthesis of bactericidal nanoparticles without using any toxic elements.

2. Materials and methods

2.1. Isolation of endophytes

Healthy plants of *C. arabica* L. were collected, washed under running tap water, and subjected to sequential surface sterilization by immersion in 3.15% sodium hypochlorite for 2 minutes, followed by 70% ethanol for 1 minute. Tissues were subsequently washed with double-distilled sterile water and dried using sterile blotter sheets. The outer tissue of surface-sterilized plant segments was excised using a sterilized scalpel, cut into 0.5–1.0-cm blocks and placed on the surface of nutrient agar supplemented with 250 μg/mL cycloheximide and incubated for 48 hours to observe colonies of endophytic bacteria [12,13]. Sterility checks were performed by transferring aliquots of final rinse water onto nutrient agar, which served as a control plate.

2.2. Screening of endophytes for synthesis of gold nanoparticles

Endophytic bacteria were cultured in nutrient medium supplemented with 1 mM gold chloroaurate and incubated at 37 °C until visible growth was observed. Colonies growing abundantly on this medium were subjected to large-scale fermentation for 72 hours under optimized conditions as described by Baker et al. [8]. The fermentation broth was centrifuged at 10,000 g at 4 °C for 5 minutes, and the supernatant was assessed for synthesis of gold nanoparticles by applying 1 mM gold chloroaurate and incubating until a color change was observed. Samples were drawn periodically and monitored using UV-visible spectrophotometry to confirm the synthesis of gold nanoparticles by recording the spectra between 200 nm and 800 nm using a Shimadzu double beam spectrophotometer (Shimadzu Corp., Kyoto, Japan).

2.3. Characterization of gold nanoparticles

Biophysical characterization of gold nanoparticles was carried out by precipitating gold nanoparticles, washing with sterile deionized water, drying under vacuum, and subjecting them to Fourier transform infrared spectroscopy (FTIR), which was carried out with a JASCO FT-IR 4100 (Jasco, Easton, MD, USA) at a resolution of 4 cm^{-1} to predict the functional group of biomolecules in the supernatant responsible for reducing metal salts and stabilizing gold nanoparticles. Diffraction pattern of the gold nanoparticles were studied by X-ray diffraction (XRD) analysis by coating the dried gold nanoparticles on a grid and recording the spectra with a Rigaku Miniflex-II Desktop X-ray diffractometer operating at a voltage of 30 kV (Rigaku, Tokyo, Japan). Size and morphology of gold nanoparticles was analyzed using transmission electron microscopy (TEM). Aliquots (~500 μL) of gold nanoparticles were transferred onto carbon-coated copper TEM grids and scanned using a JEOL JEM-2100 (Jeol, Akishima-Shi, Japan). The microscope was operated at a voltage of 120 kV with a Bioten objective lens. Subsequently, the particle size was ascertained using a Gatan CCD camera (Gatan, Pleasonton, CA, USA) [8].

2.4. Bactericidal activity of gold nanoparticles

Bactericidal activity was evaluated by well diffusion, disc diffusion and microbroth dilution assays. Prewarmed Mueller–Hinton agar plates were seeded with 10^6 colony forming units (CFU) suspensions of selected test bacteria such as *Pseudomonas aeruginosa* (MTCC 7903), *Escherichia coli* (MTCC 7410), *Staphylococcus aureus* (MTCC 7443), *Bacillus subtilis* (MTCC 121), and *Klebsiella pneumoniae* (MTCC 7407) which were swabbed uniformly. Using a sterile cork borer, a 10-mm diameter area of agar was removed and 50 μL of 10 mg/mL gold nanoparticles was added to the well, and simultaneously, the sterile agar disc was impregnated with gold nanoparticles and placed onto the agar and incubated at 37 °C for 24 hours. After incubation, the zone of inhibition was measured and interpreted with gentamicin (1 mg/mL) as standard.