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Anticancer and enhanced antimicrobial activity of biosynthesizd silver nanoparticles against clinical pathogens

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ABSTRACT

The present investigation shows the biosynthesis of eco-friendly silver nanoparticles using culture supernatant of *Enterococcus* sp. and study the effect of enhanced antimicrobial activity, anticancer activity against pathogenic bacteria, fungi and cancer cell lines. Silver nanoparticles was synthesized by adding 1 mM silver nitrate into the 100 ml of 24 h freshly prepared culture supernatant of *Enterococcus* sp. and were characterized by UV–vis spectroscopy, X-ray diffraction (XRD), Transmission Electron Microscope (TEM), Selected Area Diffraction X-Ray (SAED), Energy Dispersive X Ray (EDX) and Fourier Transform Infra red Spectroscopy (FT-IR). The synthesized silver nanoparticles were impregnated with commercial antibiotics for evaluation of enhanced antimicrobial activity. Further these synthesized silver nanoparticles were assessed for its anticancer activity against cancer cell lines. In this study crystalline structured nanoparticles with spherical in the size ranges from 10 to 80 nm and it shows excellent enhanced antimicrobial activity than the commercial antibiotics. The in vitro assay of silver nanoparticles on anticancer have great potential to inhibit the cell viability. Amide linkages and carboxylate groups of proteins from *Enterococcus* sp. may bind with silver ions and convert into nanoparticles. The activities of commercial antibiotics were enhanced by coating silver nanoparticles shows significant improved antimicrobial activity. Silver nanoparticles have the great potential to inhibit the cell viability of liver cancer cells lines (HepG2) and lung cancer cell lines (A549).

1. Introduction

Nanotechnology is rapidly growing field and deals with synthesis and control the materials with nano size ranges from 1 to 100 nm [1,2]. Size controlled nanomaterials are the foremost prerequisite of the rapidly developing field of nanomedicine used as therapeutic tools in infections against microbes in clinical application [3]. Nanoparticles are synthesized by several ways such as physical, chemical and biological methods. Among these methods, use of microorganisms is an enzymatic process. These green routes eliminate the use of expensive chemicals and are eco-friendly. Nanoparticle synthesized using microorganisms take the metal ions from their environment and convert into elemental metal through their enzymes produced by cell activities [4]. Microorganism mediated synthesis process can be classified into intracellular and extracellular synthesis according to the location where nanoparticles are produced [5]. Intracellular synthesis method means transport of metal ions into the cell by the enzymes. Extracellular synthesis method to nanoparticles is more predominant, simple and rapid reaction takes place. In this synthesis, the enzymes and proteins are present in the culture supernatant which is directly contact with heavy metals. So that the nanoparticles synthesis was occur rapidly [6].

In this study, Enterococcus sp was used to synthesis of silver nanoparticles. Enterococcus sp is a gram positive cocci shaped bacterium. Some of the Enterococcus sp was used to tannery effluent treatment to reduce the toxic level of presence of chromium. Due to this property, Enterococcus sp was selected in this study to reduce the toxic level of silver ions by converting into silver

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nanoparticles [7–10].

Nowadays many bacteria and fungi affect the people in several abnormal conditions. Commercial antibacterial and antifungal agents now in use has emerged. The microbes are resistant to these commercial antimicrobial agents while long term using for treatment of diseases. Therefore, there is an expectation and urgent need with improved medical for antibiotics with novel antimicrobial properties. Silver nanoparticles solve these limitations in the biomedical applications [11–14].

Cancer is a leading cause of disease-related mortality, and abnormal growth of cells and tissues. Cancer remains one of the world's devastating diseases and treatment includes surgical, radiation, chemotherapeutic drugs which often kills healthy cells and cause toxicity to humans. Drug design and cancer imaging are well developing due to the introduction of nanotechnology [15,16]. Anticancer activity of nanoparticles was evaluated by cell line, culture conditions for in vitro studies. Toxicity of AgNP has been studied in different cellular models such as the germ line stem cells [17] and human lung fibroblasts [18], Fibrosarcoma-Wehi 164 [19], Human Caucasian colon adenocarcinoma cell lines [20]. In this study we report the extracellular synthesis of silver nanoparticles using the culture supernatant of *Enterococcus* sp. The cytotoxicity of silver nanoparticles was studied against liver cancer cells lines (HepG2) and lung cancer cell lines (A549).

2. Materials and methods

2.1. Extracellular biosynthesis of silver nanoparticles

Microbial synthesis of metal nanoparticles depends upon the localization of the reductive components of the cell. The cell wall reductive enzymes or soluble secreted enzymes are involved in the reductive process of metal ions. Optimal condition for bacterial biomass growth was employed by using Response surface methodology using five different variables [49]. The 100 ml of optimized nutrient broth was prepared, sterilized and inoculated the fresh culture of *Enterococcus* sp. Then the culture flask was incubated for 24 h at room temperature in the orbital shaker at 120 rpm. After the incubation the culture was centrifuged at 7000 rpm for 15 min and the supernatant is collected. About 1 mM of silver nitrate was added into the supernatant of *Enterococcus* sp and incubated under the orbital shaker for synthesis of silver nanoparticles. The formation of silver nanoparticles was visually identified by following colour change and periodically monitored using UV–vis spectrophotometer at different wavelength.

2.2. Characterization studies

The microbe mediated synthesized silver nanoparticles was characterized by UV–Vis spectrophotometer at different wavelength from 360 nm–660 nm (Perkin Elmer, Lambda 25). The morphological structure of the silver nanoparticles was characterized through Transmission electron microscope (TEM). The crystalline nature of the biosynthesized nanoparticles was analysed by XRD measurement and SAED pattern. Elemental composition of synthesized silver nanoparticles was characterized by EDX spectrum. The functional groups associated with synthesized nanoparticles are analysed through Fourier Transform Infra-Red spectroscopy (FTIR).

2.3. Enhanced antibacterial and antifungal activity

2.3.1. Test bacterial and fungal strains

The six bacterial strains *Bacillus* sp. *Serratia nematodiphila* and *Streptococcus* sp. *Klebsiella pneumoniae* were obtained from Microlabs, Tamilnadu, India. *Bacillus subtilis* and *Klebsiella planticola* cultures were purchased from MTCC, Chandigarh, India. The fungal strains *Aspergillus niger*, *Aspergillus fumigatus*, *Candida* sp. and *Aspergillus flavus* were grown in Potato Dextrose Agar at 30 °C for 2 days. Fungal spore suspensions were prepared by scraping the surface of the colonies using sterile needle and the fungal spores were mixed with 10 ml sterile distilled water.

2.4. Enhanced antibacterial activity by disc diffusion method

Disk diffusion method was used to evaluate the in vitro enhanced antibacterial activity of silver nanoparticles impregnated antibiotics such as Ampicillin, Tetracycline, Novabiocin, Penicillin, Kanamycin, Gendamycin, Chloramphenicol, Streptomycin and Ciprofloxacin against clinical isolates of *B. subtilis*, *Bacillus* sp. *S. nematodiphila*, *K. planticola*, *K. pneumoniae* and *Streptococcus* sp. The standard Antibiotic discs were purchased from Hi-Media laboratories (Mumbai, India). To determine the combined effect, each standard antibiotic disc (Ampicillin, Tetracycline, Novabiocin, Penicillin, Kanamycin, Gentamycin, Chloramphenicol, Streptomycin and Ciprofloxacin) was further impregnated with 25 μL of the freshly prepared Ag-NPs.

The petriplates containing 20 ml Muller Hinton medium were seeded with 24 h culture of bacterial strains standard sterile antibiotic disks are known as a positive control, and antibiotics discs (Ampicillin, Tetracycline, Novabiocin, Penicillin, Kanamycin, Gentamycin, Chloramphenicol, Streptomycin and Ciprofloxacin) impregnated with silver nanoparticles synthesized from *Enterococcus* sp. were placed onto the MHA medium inoculated with pathogenic bacteria isolates. The inoculated plates were then incubated at room temperature for 24 h. After the incubation, the zones of inhibitions were measured and the assays were performed in triplicate.

2.5. Enhanced antifungal activity by disc diffusion method

Prepared fungal spore suspension was swabbed on Potato Dextrose Agar plates and 3 wells are made with 5 mm diameter with the help of a sterilized forceps. In this experiment, antibiotic discs, silver nanoparticles and antibiotic disc impregnated with silver nanoparticles were used to evaluate the enhanced antifungal activity. The standard antibiotic disk ketoconazole was used as control and ketoconazole disks was impregnated with 25 μL of freshly prepared silver nanoparticles were placed on the Potato Dextrose Agar plates and incubated at 37 °C for 48–78 h. A clear zone of inhibitions around the disc was examined and the diameter of zone of inhibition was measured for each organisms. The diameter of zone of inhibition was expressed in millimetre and this experiment made into triplicates to find the mean value and standard deviation.

2.6. Assessment of enhance in fold area

The enhance in fold area in zone of inhibition was evaluated by calculating the mean surface area of the inhibition zone generated by an antibiotic (a) and silver nanoparticles alone and AgNPs impregnated antibiotics (b). The fold increase area was calculated by the equation $(b^2 - a^2)/a^2$, where 'a' and 'b' refer to the zones of inhibition for antibiotic alone and antibiotic with AgNPs respectively (Birla et al., 2009).

2.7. Anticancer activity of silver nanoparticles against HepG2 and A549 cell lines

The HepG-2 and A549 cell lines were maintained at 37 °C at 5%