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Biochemical synthesis of silver nanoprticles using filamentous fungi *Penicillium decumbens* (MTCC-2494) and its efficacy against A-549 lung cancer cell line

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[ABSTRACT] Biosynthesis of silver and other metallic nanoparticles is one of the emerging research area in the field of science and technology due to their potentiality, especially in the field of nano-biotechnology and biomedical sciences in order to develop nanomedicine. In our present study, *Penicillium decumbens* (MTCC-2494) was brought from Institute of Microbial Technology (IMTECH) Chandigarh and employed for extracellular biological synthesis of silver nanoparticles. Ag-NPs formation was appeared with a dark brown color inside the conical flask. Characterization of Ag-NPs were done by UV-Spectrophotometric analysis which showed absorption peak at 430 nm determines the presence of nanoparticles, Fourier transform infrared (FT-IR) spectroscopic analysis, showed amines and amides are the possible proteins involved in the stabilization of nanoparticles as capping agent. Atomic force Microscopy (AFM) confirmed the particle are spherical, size was around 30 to 60 nm and also the roughness of nanoparticles. Field emission scanning electron microscopy (FE-SEM) showed the topology of the nanoparticles and were spherical in shape. The biosynthesis process was found fast, ecofriendly and cost effective. Nano-silver particle was found to have a broad antimicrobial activity and also it showed good enhancement of antimicrobial activity of Carbenicillin, Piperacillin, Cefixime, Amoxicillin, Ofloxacin and Sparfloxacin in a synergistic mode. These Ag-NPs showed good anti-cancer activity at 80 $\mu g \cdot mL^{-1}$ upon 24 hours of incubation and toxicity increases upon 48 hours of incubation against A-549 human lung cancer cell line and the synergistic formulation of the antibiotic with the synthesized nanoparticles was found more effective against the pathogenic bacteria studied.

[KEY WORDS] Silver nanoparticles; *Penicillium decumbens* (MTCC-2494); Antibacterial; Anti-cancer

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Introduction

Resistance developed by the pathogenic bacterial and fungal strains to commercially available antimicrobial drugs or antibiotics has become increasing alarming and has emerged as a serious menace in the recent times [1-3]. Microorganisms available in the environment are pathogenic and causes severe dysfunctions in human beings [4-5]. There is an urgent need of newer antimicrobial nanomedicine either from natural or from inorganic substances [4-6]. Silver has been employed from the ancient times to overcome infections in human as well as animals as inorganic drugs. The antibacterial efficacy of silver and its compounds has been thoroughly investigated against pathogenic bacteria and the research have shown a remarkable development in recent years [7,8]. Due to the antibiotic resistance developed by pathogenic bacteria, research in nanobiotechnology has been shifted to a new era in finding out new antimicrobial drugs to combat the multi drug resistant pathogen [9]. Nanobio-technology is an advanced technology which aims to control matter at low molecular and atomic level.In the present century nanobiotechnology is gained much attention to the researchers due to its unique properties like small size with high efficacy to the target site. Various advanced procedures like biological, chemical and physical method.are used for the biosynthesis of silver nanoparticles and the biological method offers an cost effective and ecofriendly environment[6].

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Synthesis of nanoparticles from noble metal have been studied extensively due to their magnetic, optical catalytic, antimicrobial and electronic properties[10]. Several fungi have been used for the biosynthesis of nanoparticles since they have advantage over plants and bacteria for having contained enzyme reductase, hydrogenase or electron shuttle quinone that is responsible for the reduction of silver ions in to nanosilver [11-12]. Antibacterial activity of specially formulated metal noble nanoparticles have been confirmed [15] and antimicrobial formulations based on nanoparticles could be bactericidal as an effective materials [13-15].

In the present investigation we have reported the extracellular synthesis of Silver nanoparticles from *Penicillium decumbens* (MTCC 2494). This was followed by characterization of nanoparticles and assay for antimicrobial activity against few MDRs. In the study, nanosilver were also evaluated for its antibacterial property against pathogenic bacteria (MDR) and also evaluate in combination with different antibiotics like Amoxicillin, Pipercillin, Cefixime, Carbenicillin and Ofloxacin to assess their bactericidal activity against MDRs. These nanoparticles were further checked for its anticancer activity against A-549 lung cancer cell line and cytotoxicity effect on normal Vero cells.

Materials and Methods

Penicillium decumbens (MTCC 2494) fungus was brought from Institute of Microbial Technology (IMTECH) Chandigarh, India.The fungal culture was sub cultured on Potato Dextrose Agar (PDA) medium during this study.

Synthesis of silver nanoparticles

The biomass of *P. decumbens* (MTCC-2494) was produced aerobically in a medium containing ($g \cdot L^{-1}$): yeast extract 0.6; $MgSO_4.7\ H_2O$, 0.1; glucose, 10.0; $(NH_4)_2SO_4$, 1.0; KH_2PO_4, 7.0; K_2HPO_4, 2.0 for 72 h at (25 ± 3) °C. After sufficient growth of biomass after grown the media was filtered through Whatman filter paper No. 1 and thoroughly washed 3−4 times with Milli-Q water to remove debris and media components. After washing, clean and fresh biomass of fungal species was taken into the flasks containing 100 mL of deionized Milli-Q water and incubated at 25 °C with 140 $r \cdot min^{-1}$ on orbital shaker for 72 h following incubation the biomass was sonicated for ten min and filtered through Whatman filter paper and the cell free extract was further used in the experiment. The cell free extract was added to 1 $mmol \cdot L^{-1}$ of $AgNO_3$ solution and kept in a shaker at 25 °C and 140 $r \cdot min^{-1}$ in dark condition.

Characterization of AgNPs

The change in color of the solution was observed and absorbance was measured using Cary 100 UV-visible spectrophotometer. The sample was further subjected to FT-IR analysis to determine the components for stabilization of nanoparticles. Briefly 2 mg of the sample was taken and pressed to form the thin pellet on cover slip. Sample holder was used to keep the sample and FT-IR spectra were analyzed. The sample was further characterized by AFM to check the roughness, particle size, agglomeration and shape of the nanoparticles. For AFM analysis sample was prepared by sonicating the liquid sample for 5 min followed by centrifugation at 20 000 $r \cdot min^{-1}$, made a thin film of the pellet on cover slip and subject for AFM analysis. Silver nanoparticles were further analyzed for FE-SEM analysis which is used to determine the size, shape and surface morphology of nanoparticles. For FE-SEM sample was prepared by centrifuging the liquid sample for 10 min at 20 000 $r \cdot min^{-1}$. Supernatant was discarded and pellet was dried to make it into powder form and subjected for FE-SEM analysis.

Antibacterial analysis

The multi drug resistant (MDR) pathogens were isolated and reviewed from the urine, stool and blood culture, and data were included for analysis. Then AgNPs were analyzed for its antibacterial effect using disc diffusion method[16] against various pathogenic Gram negative and Gram positive bacteria isolated from the collected sample, such as *Proteus vulgaris, Escherichia coli, Staphylococcus aureus* and *Vibrio cholera*. Infections with multi-drug resistant *Proteus vulgaris* and *E. coli*, is the leading cause of hospital-acquired and community urinary tract infections (UTIs). Twenty μg of silver nanoparticles was used along with standard antibiotic discs such as Piperacillin, Carbenicillin, Cefixime, Amoxicillin and Ofloxacin. AgNPs alone were evaluated for its antibacterial property and also in combination with Carbenicillin, Piperacillin, Cefixime, Amoxicillin and Ofloxacin to assess their bactericidal activity against pathogenic bacteria (MDR). Inhibition zone was measured after 24 h incubation at 37 °C. Experiment repeated three times and data were analyzed by standard deviation (SD) and standard error means (SEM).

Anticancer and cytotoxic activity of AgNPs.

Cell culture

The human lung cancer cell line A-549 and Vero cell line was brought from National Centre for Cell Science (NCCS) Pune India. The A-549 cells and Vero cells were separately grown in MEM as monolayer supplied with 1% glutamine, 10% fetal bovine serum (FBS), 100 $U \cdot mL^{-1}$ penicillin and 100 $U \cdot mL^{-1}$ streptomycin at 37 °C in 5% carbon dioxide (CO_2) environment.

Viability of cells

The toxicity of silver nanoparticles on human lung cancer cells (A549) and Vero cells depends on the viability of cells was evaluated by the MTT colorimetric technique. The solution of AgNPs was formed in sterile distill H_2O and just dilute it up to the certain concentrations like (20, 40, 60, 80, 100 and 120 $g \cdot mL^{-1}$) by applying the medium of cell culture The required concentration of AgNPs (*W*/*V*) of that was added to the cultured cell in the wells to obtain AgNPs final respective concentration and then incubated for 24 and 48 h at 37 °C. Alongside cells treated without AgNPS were used as