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A Novel *Streptomyces* sp. Mediated Gold Nanoparticle Synthesis and Its Efficacy on Antibiogram and Cytotoxic Activity on Breast Cancer (MCF-7) Cell Line

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Abstract: A novel *Streptomyces cavouresis* KF974778 were isolated from vermicompost by 1.5% phenol pretreatment. Gold nanoparticles (Au-Np) were synthesized from chloroauric acid using cell free supernatant of *Streptomyces cavouresis* KF974778 grown in yeast extract-malt extract broth. These nanoparticles were characterized by UV-visible spectroscopy, X-ray diffraction (XRD), Energy dispersive X-ray (EDX), Scanning electron microscopy (SEM) analysis and Transmission electron microscopy(TEM) analysis. The *Streptomyces cavouresis* KF974778 mediated Au-Np exhibited potential antibacterial activity against *E. coli, Bacillus, Streptococcus, Proteus* and *Pseudomonas* in well-diffusion method and cytotoxic activity was evaluated by MTT assay against MCF-7 breast cancer cell line confirmed that gold nanoparticles had cytotoxic activity.

Key words: Antibacterial · Cytotoxic activity · Gold nanoparticles · *Streptomyces cavouresis*

INTRODUCTION

Noble metal nanoparticles such as gold, silver and platinum are widely applied to human contacting area and there is a need to develop eco-friendly approach for nanoparticles [1]. Nobel metals have highly sensitive sensing system in healthcare, environment monitoring and probing technologies. Such metal nanoparticles have applications in drug delivery, imaging sensing and delivery of gene. Biological methods for nanoparticles synthesis using microorganism, enzyme and plant extract have been suggested as eco-friendly, compared to physical and chemical methods [2].

Actinomycetes are members of a large group of pleomorphic Gram-positive bacteria, many of which have some tendency towards mycelium growth. Actinomycetes are found in vermicompost and vermiwash, it plays an important role not only in the decomposition of organic materials and plant growth promoter, but also in their ability to produce extra cellular enzyme of pharmacological and agricultural interest [3]. Actinomycetes have high commercial values and are able to produce wide range of extracellular enzymes [4].

We have previously reported that isolation of actinomycetes from vermicompost were characterized and identified as *Streptomyces cavouresis.* 16S rRNA gene sequence is submitted in the gene bank (NCBI, USA) with accession number KF974778. The culture conditions on extracellular compound production indicated that higher biological activities were obtained when yeast extract malt extract media was used as a base. The extracellular compound of this strain has antimicrobial activity against pathogenic bacteria (*E. coli, Bacillus, Pseudomonas, Streptococcus* and *Proteus*) and fungi (*C. albicans*) [5]. The spectroscopy analysis of ethyl acetate extracellular compound from *Streptomyces cavouresis* KF974778 have shown favorable free radical scavenging activity and cytotoxic against cervical cancer (HeLa) cell line [5]. The objective of this research is extracellular synthesis of gold nanoparticles from *Streptomyces cavouresis* KF974778 and to evaluate the antibiogram and cytotoxic activity on breast cancer (MCF-7) cell line.

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MATERIALS AND METHODS

Materials: All the chemical reagents (analytical grade) were purchased from sigma-aldrich chemicals, India. The bacterial strains were obtained from Department of Microbiology, Karpagam University, Coimbatore, Tamilnadu. Bacterial cultures namely *E. coli, Bacillus, Streptococcus, Proteus*, *Pseudomonas* and were maintained in the respective medium.

Extracellular Synthesis of Au-Np: Well-grown actinomycetes culture were inoculated into 100 ml of yeast extract malt extract broth (4 g/L yeast extract, 10 g/L malt extract, 4 g/L dextrose) and incubated in rotary shaker at 28ºC for 120h. After incubation, cell free supernatant was transferred and to it was added 50 ml of 0.1 mm chloroauric acid. The whole mixture was kept in shaker at 28°C at 120 rpm. The flask was observed for visual color change from yellow to pinkish purple. Then whole mixture was centrifuged at 5000 rpm for 30 min at 4°C. The gold nanoparticles (Au-Np) were separated and were used for further studies.

Characterization of Au-Np: The absorption spectrum of Au-Np was determined in range of 200-800 nm (UV-2450, Shimadzu). For X-ray diffraction (XRD, Perkin-Elmer) analysis, thin films of Au-Np solution were prepared and the measurements were performed. The shapes of nanoparticles were analysed in scanning electron microscopy (SEM, Model JSM 6390LV, JOEL, USA). The elemental analysis was performed with energy dispersive spectrometer (EDX, RONTEC's EDX system, Model Quan Tax 200, Germany). Transmission electron microscopy (TEM) (HITACHI, H-7500) was used to determine the size and morphology of Au-Np.

Antibiogram Activity: The antibacterial activity was determined by well diffusion method [2]. The Muller Hinton Agar (2 g/L Beef extract, 17.5 g/L casein, 1.5 g/L starch, 17.0 g/L agar) were prepared and 100 µl of microbial culture was swabbed. After 5 min the well (5 mm size) was cut by gel puncher and a volume of 100 µl (25µg/ml) of the Au-Np was added into the well. The effects were compared with that of the standard antibiotic tetracycline (positive control) at a concentration of 10µg/ml. The plates were incubated at 37°C for 24 h. The assessment of the antibacterial activity was based on the measurement of the diameter of the inhibition zone formed around the well and the mean values were recorded.

Cytotoxic Activity by MTT Assay (3(4, 5-dimethylthazol-2-yl)-2-5-diphenyl Tetrazolium-bromide): Human breast cancer cell line MCF-7 (cell culture) was obtained from the National Centre for Cell Science (NCCS) Pune, India. The cells were cultured in Eagles Minimum Essential Medium (EMEM) added with FBS (10%, v/v) at 37 °C in a CO_2 incubator (95% air, 5% CO_2 and 100% relative humidity). In order to evaluate cytotoxic effect of biologically synthesized Au-Np against MCF-7 cells which were collected in the exponential stage of growth, they were seeded into 96-well plates (15,000 per well) and allowed to adhere for 48 h [5]. Then, Different concentrations (6.5, 12.5, 25, 50, 100 µg/ml) of *Streptomyces cavouresis* KF974778 mediated Au-Np were added to the desired wells and incubated for 48 h. A 20 µl of EMEM medium having MTT (5 mg/mL) was added to each well and incubated for further 4 h at 37°C. Later, the medium was altered with 100 µL of DMSO and optical densities were measured at 570nm. All studies were performed in triplicates. All the measurements were made in triplicates and expressed as the mean ± standard error (SE).

RESULTS AND DISCUSSION

Synthesis and Characterization of Gold Nanoparticles (Au-Nps): Fig. 1 shows the UV-Visible absorption spectrum of extracellular synthesized Au-Np. It has an optical absorbance range around 517 nm. The XRD analysis of Au-Np is shown in Fig. 2. The peaks at 2θ values of 38.2º and 43.5º correspond to crystal planes of (111) and (200) and thus confirmed the formation of Au-Np These phases were indexed to spherical shape. Similar report of XRD for Au-Np synthesized using *Streptomyces sp.* NK52 was found by Divya Prakash *et al*. [6].

Fig. 3 refers to the energy dispersive X-ray (EDX) analysis of Au-Np, which denotes the strong signal in the gold.

The size of biological synthesized Au-Nps was also analyzed by SEM, which is shown in Fig. 4. Particles size is in the range of 18 ± 4 nm. Balagurunathan *et al*. [7] reported that *Streptomyces* sp. mediated Au-Nps were spherical in nature and sizes ranged from 18 to 20 nm. Sadhasivam *et al*. [8] reported antibacterial activity of biosynthesized NPs from *Streptomyces hygroscopicus* against *B. subtilis, E. coli* and *E. fecalis.*

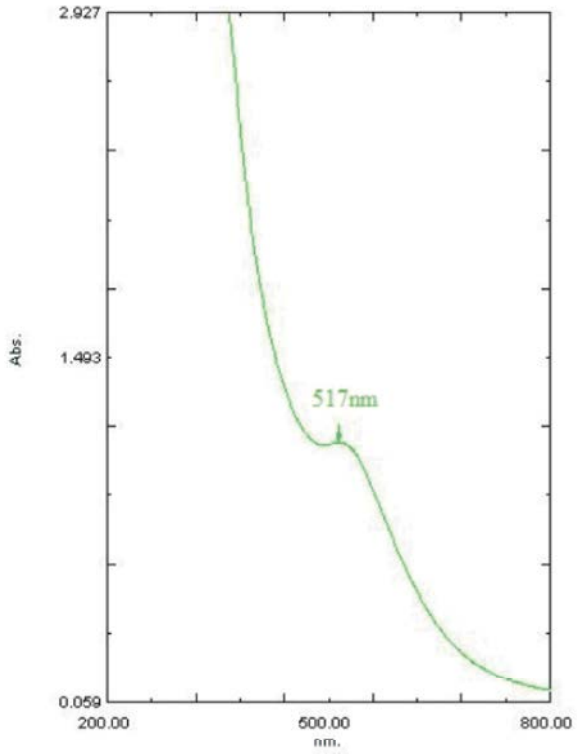

Fig. 1: UV spectra of Streptomyces cavouresis KF974778 mediated gold nanoparticles

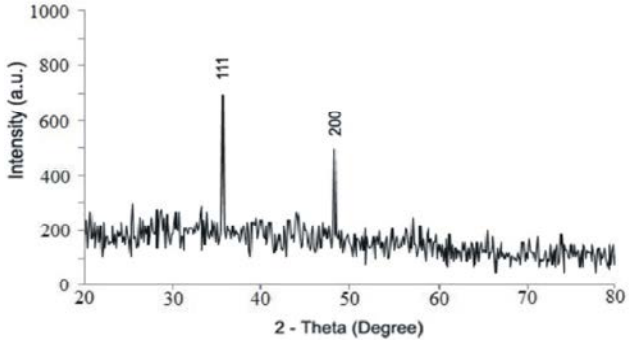

Fig. 2: XRD pattern of Streptomyces cavouresisKF974778 mediated gold nanoparticles

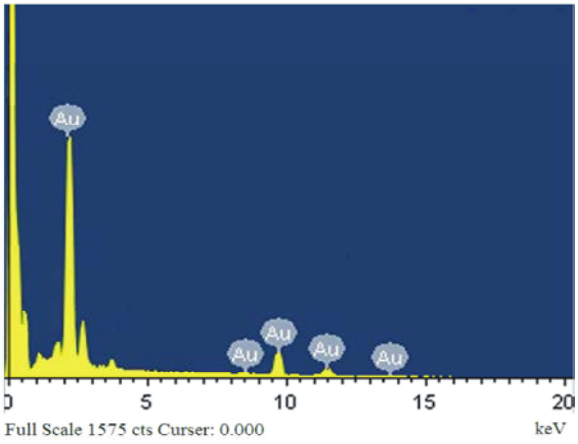

Fig. 3: EDX of Streptomyces cavouresisKF974778 mediated gold nanoparticles

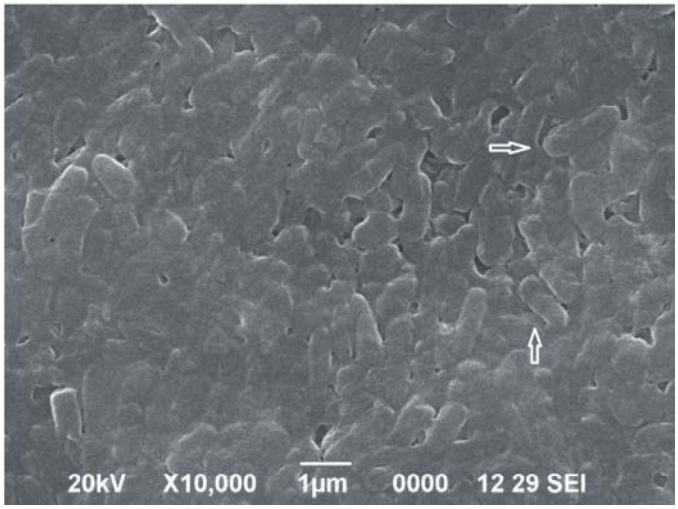

Fig. 4: SEM image of Streptomyces cavouresis KF974778 mediated gold nanoparticles

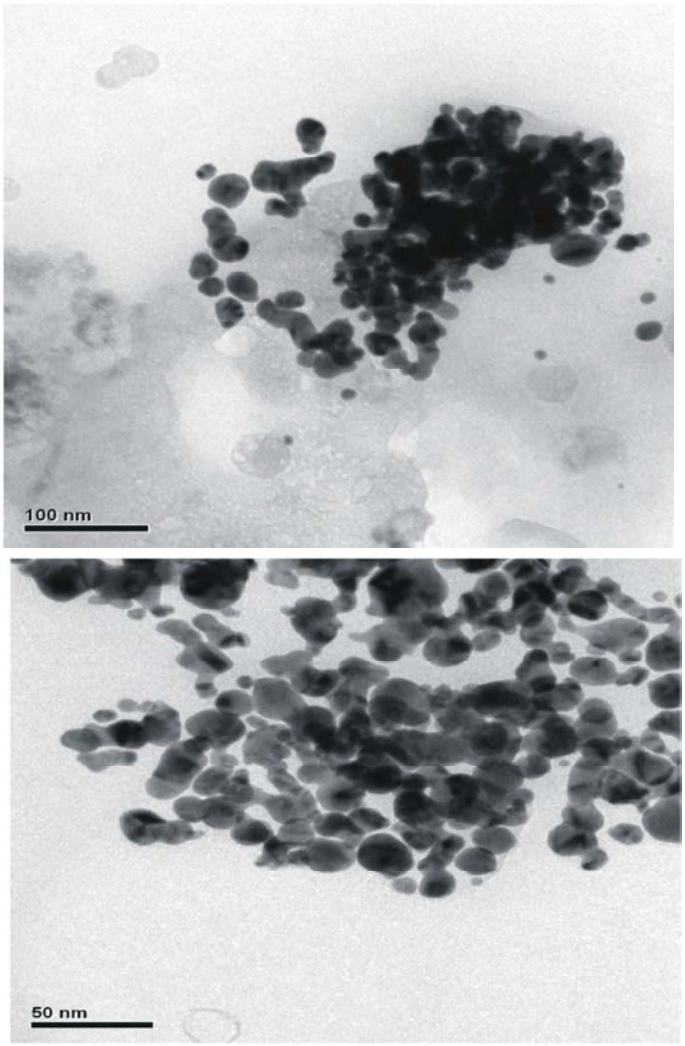

Fig. 5: HR-TEM image of *Streptomyces cavouresis* KF974778 mediated gold nanoparticles

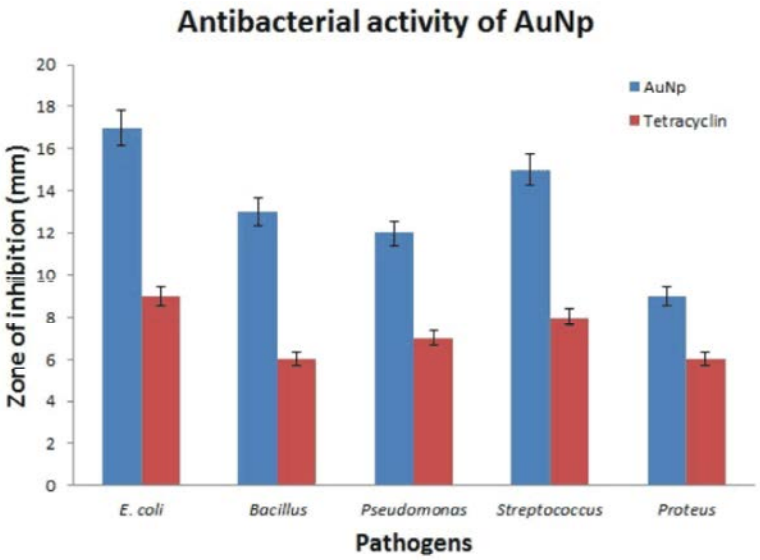

Fig. 6: Antibacterial activity of Streptomyces cavouresis KF974778 mediated gold nanoparticles

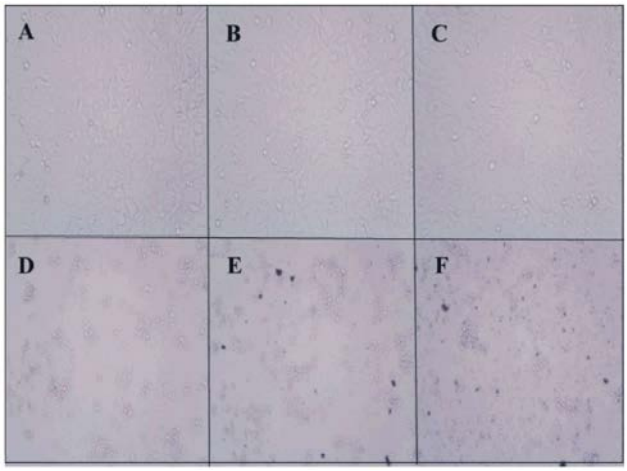

Fig. 7: Cytotoxicity effect of Streptomyces cavouresisKF974778 mediated gold nanoparticles on MCF-7 cell lines A. Control, B. 6.25 µg/ml, C. 12.5 µg/ml, D.25µg/ml, E. 50 µg/ml and F. 100 µg/ml

The resulting Au-Nps were further analyzed with TEM techniques and the average mean size of Au-Np was found to be 50 nm, which seemed to be spherical in morphology as shown in Fig. 5. HR-TEM was in good agreement with the size obtained in the SEM measurements [9].

Antibiogram Activity: Fig. 6 shows the results of antibacterial activity of *Streptomyces* sp. mediated Au-Nps against pathogenic organisms. Maximum zone of inhibition was obtained in gram negative bacteria of *E. coli* with a zone diameter of 17 ± 2 mm at a concentration of 25 µg/ml and lowest zone of inhibition was observed in *Proteus* with a zone diameter of 9 ± 1 mm at the same concentration. Balagurunathan et al. had earlier studied the antibacterial activity of Au-Nps against both gram-positive and negative bacterial strains [7] Shirley *et al*. [10] reported that Au-Nps had bactericidal effect against *Eschericia coli, Pseudomonas aeruginosa, Proteus and Streptococcus.* The growth inhibition of cell may be due to disruptions of cell membrane by Au-Nps resulting in breakdown of cell enzyme [11]. The current results revealed that *Streptomyces cavouresis* KF974778 mediated Au-Nps had effective antibacterial activity.

Cytotoxicity Studies: The cytotoxicity effect of the Au-Nps was evaluated against MCF-7 breast cancer cell line at a various concentrations (6.5- 100 µg/ml). Fig. 7

shows the cytotoxicity activity of Au-Nps The IC_{50} value for Au-Nps was 49.82 µg/ml. Maximum concentration of Au-Nps (100µg/ml) effectively inhibits the growth of cell by more than 93%. Selim and Hendi [12] had earlier reported the anticancer activity of Au-Np and cytotoxic effects of Au-Nps against MCF-7 human breast cancer cells.

CONCLUSIONS

This study concludes that vermicompost derived Streptomyces cavouresis KF974778 mediated Au-Nps synthesis can be a prominent source for the development of various nano medicines.

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