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Controlling of disease causing pathogens using silver nanoparticles synthesized by one step green procedure

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ABSTRACT

Biosynthesis of nanoparticles is the important area in applicable nanoparticles and development of nanobiotechnology. In this present investigation, we used soil bacteria *Proteus* sp for the biosynthesis of silver nanoparticles. The synthesized nanoparticles were visually observed and characterized using UV-vis spectrophotometer for its surface plasmn resonance, crystalline nature was identified by X-ray diffraction assay and morphology was identified using scanning electron microscope. The peak at 430 nm in UV-vis spectroscopy confirms the SPR and XRD shows the intensity of (1 1 1) and (2 2 2) confirm the crystalline nature. The 50-100 nm sized and spherical shaped nanoparticles are synthesized was confirmed by SEM. The antibacterial activity of the silver nanoparticles analysed using agar well diffusion method, shows very good zone of inhibition equals to commercially available antibiotics.

INTRODUCTION

Biosynthesis of nanoparticles is the emerging field in nanoscience and nanotechnology. Using of biological entities such as bacteria, fungus, yeast, actinomycetes, plants and algae for the synthesis of silver nanoparticles have been developed. The bacterial isolates such as Marine bacteria *Enterococcus* sp. (Rajeshkumar *et al*., 2016), extremophilic *Ureibacillus thermosphaericus* (Juibari *et al*. 2011), *Gluconobacter roseus* (Krishnaraj and Berchmans, 2013), *Vibrio alginolyticus* (Rajeshkumar *et al*., 2013), *Pseudomonas aeruginosa* (Kumar and Mamidyala 2011), *Bacillus* sp. (Malarkodi *et al*., 2013), *Bacillus licheniformis* (Kalimuthu *et al*., 2008) , *Enterobacter aerogenes* (Karthik and Radha, 2012), *Streptomyces* sp. LK3 (Karthik *et al*., 2014), *Escherichia coli* ATCC 8739, *Bacillus subtilis* ATCC 6633, and *Streptococcus thermophiles* ESh1 (El- Shanshoury *et al*., 2011), *Serratia nematodiphila* (Malarkodi *et al*., 2013), *Bacillus subtilis* MTCC 3053 (Paulkumar *et al*., 2013), *Acinetobacter calcoaceticus* (Gaidhani *et al*., 2013), *Salmonella typhirium* (Ghorbani, 2013) *Brevibacterium casei* (Kalishwaralal *et al*., 2010), thermophilic bacterium *Geobacillus stearothermophilus* (Fayaz *et al*., 2011). Silver nanoparticles are having wide range of applications in biomedical arena such as antibacterial activity against both gram positive and gram negative bacterial isolates (Rajeshkumar, 2016) and food borne pathogens (Rajeshkumar and Malarkodi, 2014), antifungal activity against *Aspergillus niger, Aspergillus fumigatus, Candida albicans, Aspergillus flavus* and *Fusarium* sp (Rajeshkumar *et al*., 2014), anticancer activity against liver and lung cancer lines, enhanced antibacterial (increasing of antibiotics sensitivity by coating of silver nanopartilces with different antibiotics disc such as Ampicillin, Tetracycline, Novabiocin, Penicillin, anamycin, Gendamycin, Chloramphenicol, Streptomycin and Ciprofloxacin) effect (Rajeshkumar *et al*., 2016). Catalysis (Jiang *et al*., 2005), biosensors for antigen and antibody binding (Zhu *et al*., 2009), controls the growth the dermatophytes, antiviral activity against

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human immuno deficiency, virus, Molecular imaging of cancer cells, wound healing properties (Caro *et al*., 2010). In this present investigation we used *Proteus* sp isolated from soil sample for the synthesis of silver nanoparticles and it was characterized using UV-vis spectrophotometer, Scanning electron microscope, elemental analysis, X-ray diffraction assay. The antibacterial activity of silver nanoparticles against *Staphylococcus aureus, Escherichia coli, Bacillus sp, Salmonella sp, pseudomonas areogenusa* and *Klebsiella pneumoniae* was performed.

MATERIALS AND METHODS

Isolation and Identification of bacteria

1g soil sample was added into 100 ml sterile distilled water and agitated for uniform microbial suspension. Serial dilutions were performed by decimal dilutions made up to 10^{-7}. From these dilutions 1 ml aliquots were poured into sterile petriplates containing Nutrient Agar medium (Hi – media, Mumbai, India).

Plates were incubated at 37 ºC for 24-48 hours. The individual colonies were isolated by streaking on nutrient agar plates and incubated at 37 °C for 24 hrs and the bacteria were identified based on morphological structure. The morphological and physiological characterization of the isolate was performed according to the methods described in Bergey's Manual of determinative bacteriology.

Synthesis and characterization of silver nanoparticles

In extracellular synthesis process, the culture supernatant of *Proteus* sp was used. The culture was grown in nutrient broth. *Proteus* sp was grown in 250 ml conical flask containing 100 ml of sterile nutrient broth for 24 hours at 37 °C in rotary shaker at 220 rpm. After the incubation the culture was centrifuged at 10,000 rpm for 10 minutes and collects the supernatant. To this supernatant 1 mM of silver nitrate was added and change of colour was noted at 24 h of incubation.

The change of colour was indicates the formation of silver nanoparticles and periodically analysed by UV-vis Spectrophotometer at different wavelength. The silver nanoparticles powder was prepared using centrifugation and it was washed 3 times using double distilled water. Finally the collected pellets was collected in the petri-plates and kept in hot air oven for 80 °C. The nanoparticles powder was characterized using SEM, EDX and XRD.

Antibacterial activity

Luria Bertani Agar medium was used to cultivate bacteria. Fresh overnight culture of each strain was swabbed uniformly onto the individuals plates using sterile cotton swabs. 3 wells were made on each Luria Bertani Agar plates. Then the centrifuged silver nanoparticles (25 µl, 50 µl and 75µl) were poured into each well on all plates and incubate for 24 hrs at 37 °C. After incubation the different levels of zonation formed around the well was measured.

RESULTS AND DISCUSSION

Synthesis of silver nanoparticles using *Proteus* sp.,

Extracellular synthesis of silver nanoparticles using *Proteus sp* was primarily identified by visual identification, appearance of whitish brown colour in the reaction mixture indicates the formation of nanoparticles **(Fig 1).** The bacterial biomass of culture shows pale yellow in colour before the addition of silver nitrate and this changed into light brown colour. The colour was formed after the 12 h of incubation indicating the rapid preparation of silver nanoparticles. After 48 h of incubation the reaction mixture shows brownish colour indicating the completion of reaction. The formation of brownish colour in bacterial biomass indicates the silver nanoparticles (Rajeshkumar *et al*., 2016).

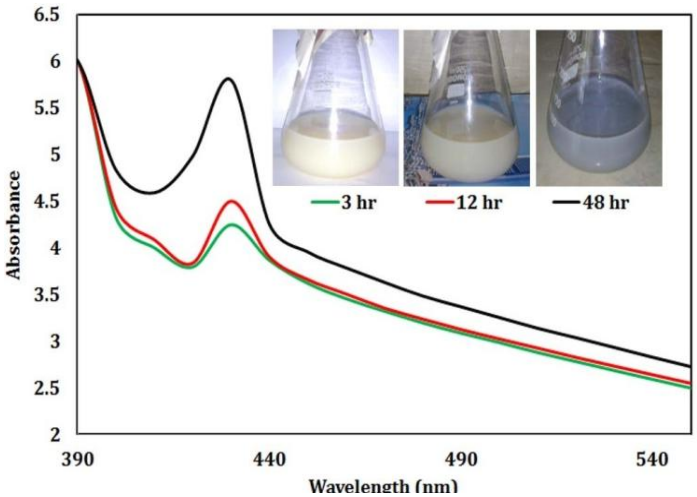

Fig. 1: UV-vis absorbance and visual observation of biosynthesis of silver nanoparticles using *Proteus sp.*

Fig. 1 shows the UV spectrum recorded as synthesized silver nanoparticles at different functional time. Paulkumar *et al*., 2014 reported that UV-vis Spectroscopy is a very useful technique to characterize the silver nanoparticles synthesis. The culture supernatant of *Proteus* sp., was collected at the stationary phase for extreme nanoparticles biosynthesis. UV- vis spectrophotometer shows that the silver nanoparticle synthesis after the adding of silver nitrate into the bacterial culture at the growth phase, the absorption band was formed at 430 nm at the incubation time of 3 h with small peak indicates the formation of small size of nanoparticles. After the 12 h of incubation the absorption peak was shift into 429 nm due to the excitation of surface plasmon resonance of the nanoparticles. The alterations in the position of surface plasmon resonance band due to the development of differences in the shape or size of nanoparticles by the biogroups of *Serratia nematodiphila* and *Bacillus subtilis* (Malarkodi *et al* 2014; Paulkumar *et al* 2014). We recorded the absorbance on 48 hr shows peak at 430 nm indicates the stability of the nanoparticles.

X-Ray Diffraction assay

Fig 2 shows the XRD spectrum of bio synthesized silver nanoparticles using culture supernatant of *Proteus sp* . The XRD pattern showed the whole spectrum of 2 theta scale ranges from 10-80. The two intense peaks showed at 2 theta scale values of 38.0° and 44.0° corresponding to the planes of (1 1 1) and (2 2 2) for fcc of silver. The strong peak at 38.0° and 44.0° is ascribed of face-centered cubic silver structure, while other diffraction peak shows much weak. The sharpening of the peaks evidently indicates that the particles are in the size of nano (Amin *et al*. 2013, Penga *et al*., 2013). The weak peaks are due to the organic moiety present in the bacterial culture (Pourali, and Yahyaei, 2016).

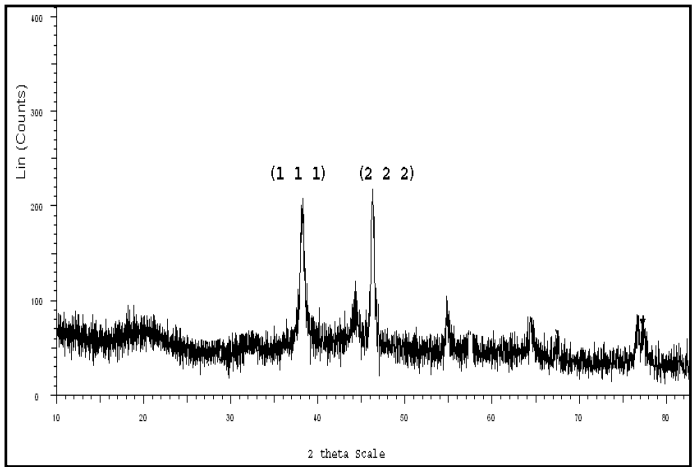

Fig. 2: X-ray diffraction analysis of biosynthesized silver nanoparticles.

Scanning electron microscope

Morphological characterization of silver nanoparticles synthesized by using *Proteus sp* was analysed using SEM shown in fig 3. The nanoparticles show undefined spherical and rectangle shape with high agglomeration. The size of the nanoparticles ranges from 50-100 nm magnifications. Agglomeration found due to higher proportion of capping agent in the bacterial biomass. The reduced amount of organic moieties also responsible for the agglomeration of nanoparticles (Paulkumar *et al*., 2014).

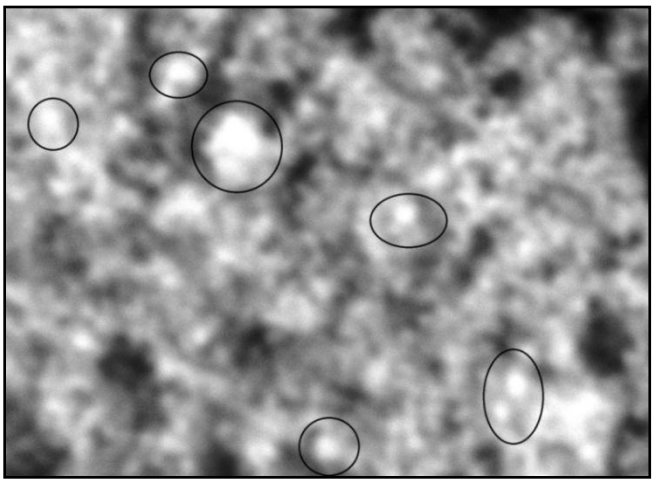

Fig. 3: SEM image of silver nanoparticles synthesized using *Proteus* sp.

Energy dispersive X-ray spectroscopy

Analysis using Energy dispersive X-ray (EDX) spectrometer confirms the presence of elemental silver signal of the silver nanoparticles **(Fig 4).** Documentation lines for the major emission energies for silver (Ag) are displayed and these are similar to with peaks at 3keV in the spectrum, thus giving confidence that silver has been correctly identified. EDX analysis confirmed the elements were Ag and O and the weight percentage were 63.70% and 36.30% respectively. Some of the weak peaks also observed in EDX are formed due to X-ray emission from various organic moieties present within the bacterial culture.

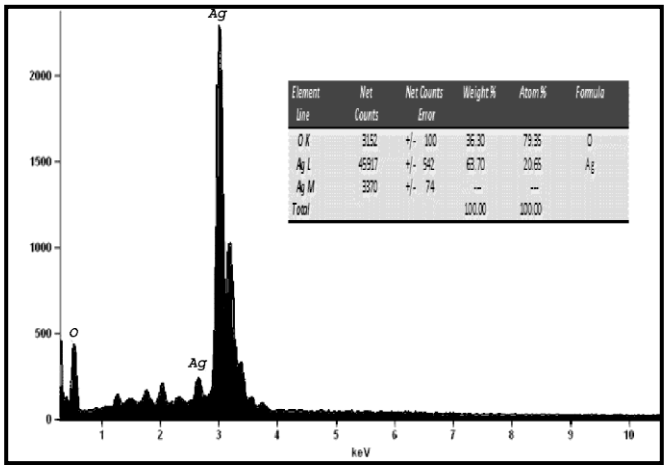

Fig. 4: EDX spectrum of silver nanoparticles synthesized from *Proteus* sp.

Antibacterial activity of nanoparticles

Antibacterial activity of silver nanoparticles against gram positive bacteria like *Bacillus* sp and *Staphylococcus aureus* and gram negative isolates such as *Salmonella sp, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *E.coli* performed by agar well diffusion method. The bacterial cultures we used are mostly disease causing pathogens like Salmonella is causative agent for typhoid fever, *E.coli* and *S. aureus* are food borne pathogens and other bacterial strains are responsible so many infections to plants and humans. The silver nanoparticles are sensitive against all the pathogens, in that when compare to gram positive gram negative bacteria have better zone of inhibition (Fig 5 & 6).

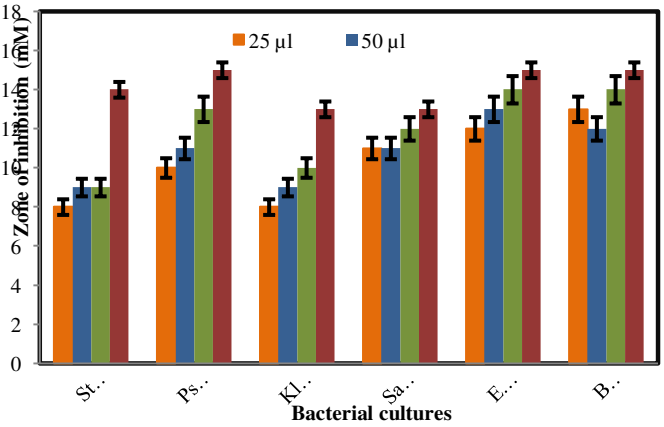

Fig. 5: Clustered column chart of antimicrobial activity of silver nanoparticles.

Among the four gram negative pathogens the silver nanoparticles very effectively work against *Pseudomonas aeruginosa* is a multidrug resistant pathogen. Silver nanoparticles may disturb the cell wall of the bacteria and involved in the process of inhibition of DNA replication is major reason for controlling of bacterial growth (Rajeshkumar, 2016).

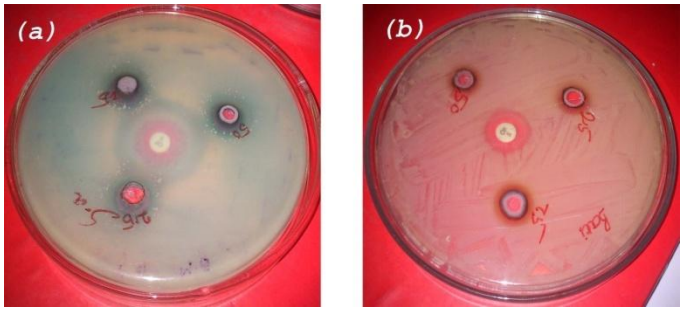

Fig. 6: Antimicrobial activity of silver nanoparticles against gram positive bacteria (a) *Staphylococcus aureus* and (b) *Bacillus* sp.

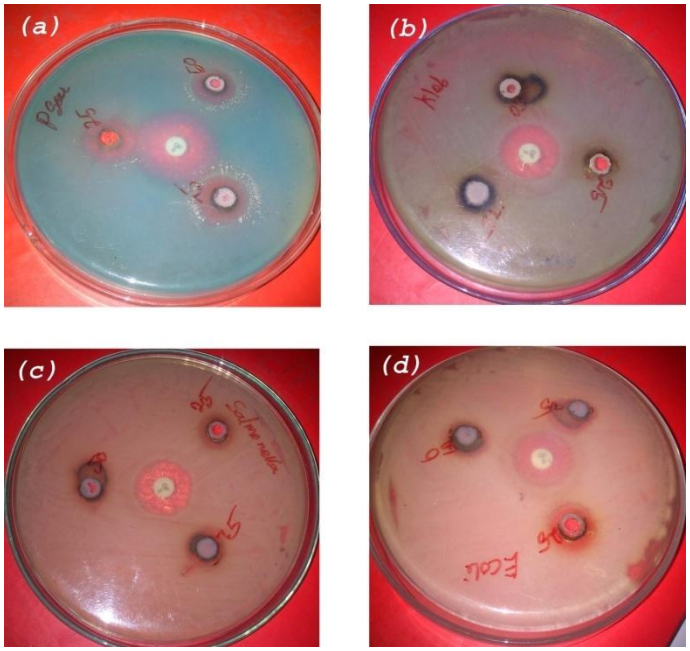

Fig. 7: Antimicrobial activity of silver nanoparticles against gram negative bacteria (a) *Pseudomonas aeruginosa* (b) *Klebsiella pneumoniae (c) Salmonella sp* and *(d) E.coli.*

CONCLUSION

In this present study we used *proteus sp* a soil bacteria for the synthesis of silver nanoparticles using one step synthesis. The results of UV-vis spectroscopy, XRD, SEM and EDX are confirm the surface plasmon resonance, very good crystalline nature, spherical shape and elements of bacterial meditaed synthesis of silver nanoparticles. The stability of the nanoparticles is good was confirmed by UV-vis spectroscopic analysis. The silver nanoparticles have good growth control of some disease causing pathogens may responsible for food poisoning, typhoid fever, pneumonia fever and etc. Based on our results, it is possible to produce pharmaceutical agents and drugs for the above mentioned health problems.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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