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# Antibacterial activity of silver nanoparticles synthesized extracellularly by soil micro flora

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**Abstract**

The microbes used to synthesize the silver nanoparticles were isolated from the soil and identified as E.coli, Pseudomonas sp, Aspergillus sp, Penicillium sp and Actinomycetes sp. All the isolated organisms were cultured in broth medium and used for the synthesis of silver nanoparticles extracellularly by adding 3 -10mm AgNO3. The presence of extracellular silver nanoparticles were identified by UV – visible spectrophotometer (380-420nm) and particle size investigation. Most of the microbial synthesized products are susceptible for Proteus sp. Bioactive particles from E.coli and Actinomycetes sp. were found to be best against Klebsiella sp. The active molecule from Pseudomonas sp and Penicillium sp. showed very good sensitivity pattern against Proteus sp. The molecules formed by Aspergillus sp. showed higher sensitivity against Enterobacter sp. Among the three bacterial and two fungal species, the silver nanoparticles from the fungal organisms showed the better result against pathogens.

**Keywords:** Bacteria, Fungi, Pathogens, Antimicrobial Activity, Silver Particles.

# Toprak mikroflora tarafından ekstraselüler sentezlenen gümüş nanoparçacıklar antibakteriyel aktivitesi

**Özet**

Gümüş nanopartiküllerini sentezlemek için kullanılan mikroplar toprak ortamından izole edilmiş ve E.coli, Pseudomonas sp, Aspergillus sp, Penicillium sp and Actinomycetes sp olarak tanımlanmıştır. İzole edilen bütün organizmalar et suyu ortamında kültürlenmiş ve 3 -10mm AgNO3 eklenerek hücre dışı gümüş partiküllerin sentezi için kullanılmıştır. Hücre dışı gümüş partiküllerin varlığı, UV Vis (380-420nm)ve partikül boyut incelemesiyle belirlenmiştir. Mikrobiyal sentezlenen ürünlerin çoğu, Proteus sp.'ye duyarlıdır. E.coli and Actinomycetes sp.'den kaynaklanan biyoaktif partiküller Klebsiella sp.'ye karşı en iyi olarak bulunmuştur. Pseudomonas sp ve Penicillium sp türlerinden kaynaklanan aktif molekül Proteus sp'ye oldukça duyarlı bir durum sergilemiştir. Aspergillus sp. Tarafından oluşan moleküller Enterobacter sp.'ye karşı yüksek duyarlılık göstermiştir. Üç bakteri ve iki mantar türleri arasında mantarlardan kaynaklanan gümüş partiküller patojenlere karşı daha iyi sonuç vermiştir.

**Anahtar Kelimeler**

## 1. Introduction

Nanoscience is currently a fast growing niche and nanotechnology is at the cutting edge of this rapidly evolving area[1]. Nanotechnology collectively describes technology and science involving nano scale particles (nanoparticles) that increases the scope of investigating and regulating the interplay at cell level between synthetic materials and biological systems [2]. Most of the natural processes also like place in the nanometer scale regime. Therefore a confluence of nanotechnology and biology can address several biomedical problems and can revaluate in the field of health and medicine.

Synthesis of nanoparticles of different shapes and size is an emerging area of research due to their use in a variety of biological fields.

To date metallic nanoparticles are mostly prepared from Nobel metals (i.e., Ag, Pt, Au and Pd). The use of metallic nanoparticles in the field of catalysis, optoelectronics, diagnostic biological problems and display devices uncovered many significant findings. Among the Nobel metals, silver (Ag) is the metal of choice in the field of biological systems, living organisms and medicine[3].

Silver nanoparticles are nanoparticles of silver, i.e. silver particles of size between 1nm and 100nm in size. While frequently described as being 'silver' some are composed of a large percentage of silver oxide due to their large ratio of surface to bulk silver atoms. The reduction of Ag+ ions by combinations of bio molecules found in these extracts such as vitamins, enzymes/proteins, organic acids such as citrates, amino acids, and polysaccharides [4]. The ability of some micro organisms such as bacteria and fungi to control the synthesis of metalic nanoparticles should be employed in the search for new materials.

Silver is highly toxic to most microbial cells and can be used as biocide or antimicrobial agent nevertheless, it has been reported that several bacterial strains ace silver resistant [5 & 6]. Silver nanoparticles may have an important advantage over conventional antibiotics in that they kill all pathogenic microorganisms [7,8 & 9].

The synthesis of silver nanoparticles by the reduction of aqueous Ag+ ion by simultaneous reduction of aqueous Ag+ with the culture broth of some tested bacteria and fungi. Through Kroger et al., [10] screening process involving a number of bacteria they observed that Enterobacteria group were potential candidate for rapid synthesis of silver nanoparticles.

The extracellular synthesis of nanoparticles from Fusarium oxysporum [11] Pseudomonas stutzeri AG259 [12] and Penicillium sp. [13] were studied. The genus Penicillium seems to have extremely good candidates for the fabrication of Ag NPS. In this study, we performed the synthesis of silver nanoparticles from different groups of microorganisms and its antimicrobial activity against some human pathogens.

## 2. Materials and Methods

### 2.1. Isolation and characterization of silver nano producing microorganism

The soil samples were collected from the garden in various places. The colonies with different cultural characteristics were selected for the study. The selected colonies were identified by Bergey's manual [14].

### 2.2. Collection of Pathogens

The pathogens such as Proteus sp., Klebsiella sp., Staphylococcus sp. and Enterobacter sp. used for the antimicrobial activity were collected from Vivek Laboratory, Nagercoil.

### 2.3. Preparation of cell free microbial extract

The bacterial and fungal biomass used for biosynthetic experiments were grown aerobically in liquid medium containing (g/l): KH2PO4 K2HPO4 2.0, MgSO4 . 7H2O 0.1, (NH4)2SO4 10, yeast extract 0.6, glucose 10.0. The liquid medium was prepared, sterilized and inoculated with fresh culture of the test strains. The cultured flasks were incubated at 25oC with shaking (150rpm) for 72 hrs. After incubation the cultures were centrifuged at 12,000 rpm and the supernatants were used for the synthesis of silver nanoparticles.

### 2.4. Preparation of silver nanoparticles from microbes

The bacterial and fungal biomass used for biosynthetic experiments were grown aerobically in liquid medium after incubation time the cultures were centrifuged at 12000 rpm and their supernatants were used for further experiments. Silver nitrate at concentration of 10-30 mm was separately added to the reaction vessels containing different supernatants (1% v/v). The reaction between supernatants and Ag+ ions were carried out in the dark condition. After the development of the brown colour, aliquots of the reaction solution were removed and the absorptions were measured using a UV - visible spectrophotometer. Furthermore, the silver

nanoparticles were analyzed by particle size analyzer.

### 2.5. Preparation of disc

The sterile discs approximately 5mm in diameter was placed on MHA plates treated with different concentration of Ag nano particles. The disc was then placed over the swabbed MHA plates and incubated for 24 hrs at 37oC to study the antimicrobial activity

### 2.6. Antimicrobial activity of silver nanoparticles against pathogens

The antimicrobial susceptibility of sliver nanoparticles was evaluated using Kirby- Bauer method. Zones of inhibition were measured after 24hrs of incubation. The comparative stability of discs containing control was made.

## 3. Results And Discussion

The microorganisms were identified as E.coli, Pseudomonas sp., Actinomycetes sp., Aspergillus sp. and Penicillium sp.

The Erlenmeyer flasks with E.coli, Actinomycetes sp., Pseudomonas sp., Aspergillus sp., and Penicillium sp., supernatants were a pale yellow colour before the addition of Ag+ ions and this changed to a brownish colour on completion of the reaction with Ag+. The appearance of a brown colour in solution suggested the formation of silver nanoparticles. Thus, it was evident that the metabolites excreted by the culture exposed to silver could reduce silver ions, clearly indicating that the reduction of the ions occur extracellularly through reducing agents released in the solution by Actinomycetes sp., Pseudomonas sp., Aspergillus sp., and Penicillium sp. The silver nanoparticles were characterized by UV - Visible spectroscopy. This technique has proved to be a very useful technique for the analysis of nanoparticles.

UV-Visible spectra for the absorbance spectrum of silver nanoparticles synthesized by E.coli, Actinomycetes sp., Pseudomonas sp., Aspergillus sp., and Penicillium sp., the strong, but broad surface Plasmon peak located in 399nm(2.1305), 390.5nm(1.0648), 393.5nm (1.1302), 387.0nm(2.8294) and 390.5nm(1.4205) respectively. Biologically synthesized silver nanoparticles could have many applications in area such as non-liner optics, spectrally selective coating for electrical batteries, optical receptors, catalysis in chemical reactions, bio labeling [15] and antibacterial capacity [16].

The antibacterial activity of silver nano particles from E.coli was given in Table 1.

**Table 1**. Antibacterial activity of Silver nano particles from *E.coli*

| Concentration of silver nano particles from cultures (µl/disc) | Zone of Inhibition (mm) | | | |
|---|---|---|---|---|
| | *Enterobacter* sp. | *Staphylococcus* sp. | *Proteus* sp. | *Klebsiella* sp. |
| 100 | 10.30±0.26 | 8.40±0.20 | 8.23±0.15 | 8.20±0.10 |
| 200 | 10.66±0.15 | 8.63±0.25 | 10.16±0.15 | 8.26±0.15 |
| 300 | 12.70±0.12 | 10.30±0.10 | 10.30±0.10 | 8.46±0.15 |
| 400 | 12.80±0.10 | 10.50±0.10 | 12.23±0.20 | 10.20±0.20 |
| 500 | 14.33±0.30 | 10.63±0.20 | 12.46±0.32 | 10.52±0.26 |

Among the different silver nanoparticles, the best result obtained from E.coli culture was 14.33 ± 0.3mm zone of inhiibition at 500µl/disc concentration against Enterobacter sp. The antibacterial activity was limited in Klebsiella sp. was 10.50 ± 0.26mm zone around the colonies at 500µl/disc concentration. For Staphylococcus sp. and Proteus sp. showed 10.63± 0.20mm and 12.46 ± 0.32mm zone of inhibition at 500µl/disc concentration respectively. Antimicrobial activity of Ag nanoparticles from microbes against

Enterobacter sp., Staphylococcus sp., Proteus sp., and Klebsiella sp. showed related result to that found by Sondi and Salopek-Sondi, [16].

The antibacterial activity of silver nano particles from Pseudomonas sp was reported in Table 2.

**Table 2.** Antibacterial activity of Silver nano particles from *Pseudomonas* sp.

| Concentration of silver nano particles from cultures (µl/disc) | Zone of Inhibition (mm) | | | |
|---|---|---|---|---|
| | *Enterobacter* sp. | *Staphylococcus* sp. | *Proteus* sp. | *Klebsiella* sp. |
| 100 | 12.50±0.10 | 8.30±0.52 | 22.23±0.20 | 14.40±0.10 |
| 200 | 14.30±0.26 | 12.60±0.15 | 22.63±0.15 | 18.26±0.25 |
| 300 | 14.63±0.15 | 10.61±0.23 | 24.20±0.10 | 22.40±0.36 |
| 400 | 16.63±0.30 | 15.34±0.26 | 24.53±0.11 | 24.46±0.15 |
| 500 | 16.66±0.05 | 15.30±0.10 | 24.76±0.15 | 24.70±0.10 |

The silver nanoparticles prepared from Pseudomonas sp. showed 24.76 ± 0.15mm inhibition zone at 500µl/disc concentration against Proteus sp. The antibacterial activity were limited in Staphylococcus sp. was 15.30 ± 0.10mm zone at 500µl/disc concentration and for pathogens, Klebsiella sp. and Enterobacter sp. the activity was found to be 24.70 ± 0.10mm and 16.60 ± 0.05mm zone of inhibition at 500µl /disc concentration respectively.

The antibacterial activity of silver nano particles from Actinomycetes sp. was shown in Table 3.

**Table 3**. Antibacterial activity of Silver nano particles from *Actinomycetes* sp.

| Concentration of silver nano particles from cultures (µl/disc) | Zone of Inhibition (mm) | | | |
|---|---|---|---|---|
| | *Enterobacter* sp. | *Staphylococcus* sp. | *Proteus* sp. | *Klebsiella* sp. |
| 100 | 8.06±0.11 | 8.10±0.10 | 12.10±0.10 | 8.33±0.10 |
| 200 | 8.20±0.20 | 8.20±0.20 | 12.36±0.05 | 12.61±0.25 |
| 300 | 8.33±0.05 | 8.40±0.10 | 12.50±0.10 | 10.6±0.10 |
| 400 | 8.50±0.10 | 8.63±0.20 | 12.63±0.11 | 15.30±0.20 |
| 500 | 8.73±0.05 | 8.83±0.11 | 14.26±0.25 | 15.31±0.52 |

The silver nanoparticles synthesized from Actinomycetes sp. culture showed 15.31±0.52mm zone of inhibition at 500µl/disc concentration against the pathogen, Klebsiella sp. The antibacterial activity was limited in Proteus sp., Staphylococcus sp., Enterobacter sp. such as, 14.26±0.25mm, 8.83±0.11mm and 8.73±0.05mm zone of inhibition at 500µl/disc concentration respectively.

The antibacterial activity of silver nano particles from Aspergillus sp. was given in Table 4.

**Table 4**. Antibacterial activity of Silver nano particles from *Aspergillus* sp.

| Concentration of silver nano particles from cultures (µl/disc) | Zone of Inhibition (mm) | | | |
|---|---|---|---|---|
| | *Enterobacter* sp. | *Staphylococcus* sp. | *Proteus* sp. | *Klebsiella* sp. |
| 100 | 8.20±0.15 | 8.10±0.10 | 8.20±0.10 | 10.56±0.15 |
| 200 | 8.56±0.15 | 8.20±0.20 | 8.40±0.10 | 14.10±0.26 |
| 300 | 8.40±0.10 | 8.56±0.15 | 8.53±0.11 | 14.10±0.26 |
| 400 | 12.61±0.20 | 10.30±0.26 | 10.66±0.20 | 15.30±0.20 |
| 500 | 22.30±0.52 | 10.50±0.10 | 14.30±0.26 | 15.30±0.20 |

Silver nanoparticles prepared from the fungi, Aspergillus sp. showed 22.30±0.52mm zone around the colonies at 500µl /disc concentration against the pathogen, Enterobacter sp. The antibacterial activity found against Klebsiella sp., Proteus sp. and Staphylococcus sp. was 15.30±0.20mm, 14.30±0.26mm and 10.50±0.10mm zone of inhibition at 500 µl /disc concentration respectively.

The antibacterial activity of silver nano particles from Penicillium sp. was given in the Table 5.

**Table 5**. Antibacterial activity of Silver nano particles from *Penicillium* sp.

| Concentration of silver nano particles from cultures (µl/disc) | Zone of Inhibition (mm) | | | |
|---|---|---|---|---|
| | *Enterobacter* sp. | *Staphylococcus* sp. | *Proteus* sp. | *Klebsiella* sp. |
| 100 | 8.10±0.10 | 8.43±0.15 | 14.30±0.26 | 8.30±0.10 |
| 200 | 8.36±0.05 | 8.70±0.10 | 14.60±0.10 | 12.60±0.26 |
| 300 | 8.53±0.11 | 10.20±0.10 | 14.83±0.05 | 12.61±0.28 |
| 400 | 8.70±0.10 | 10.60±0.20 | 26.10±0.10 | 15.30±0.20 |
| 500 | 10.26±0.25 | 10.76±0.15 | 30.16±0.20 | 26.10±0.10 |

The silver nanoparticles from Penicillium sp. was 30.16±0.20mm zone of inhibition at 500 µl /disc concentration against the pathogen Proteus sp. The antibacterial activity were limited in Enterobacter sp. 10.26±0.25mm at 500 µl /disc concentration and for Staphylococcus sp. and Klebsiella sp. antibacterial activity was observed as 10.76±0.15mm and 26.10±0.10mm zone at 500 µl /disc concentration respectively.

The nanoparticles from the various microorganisms, E.coli, Pseudomonas sp., Actinomycetes sp., Aspergillus sp. and Penicillium sp. showed best activity against the pathogens. Most of the microbial synthesized products are sensitive for Proteus sp. Bioactive particles from E.coli and Actinomycetes sp. were found to be best against Klebsiella sp. The active molecule from Pseudomonas sp and Penicillium sp. showed very good sensitivity pattern against Proteus sp. The molecules formed by Aspergillus sp. showed higher sensitivity against Enterobacter sp (Table 6).

**Table 6**. Antibacterial activity of Silver nano particles synthesized from soil microbes against pathogens

| Concentration of silver nano particles from cultures (500µl/disc) | Zone of Inhibition (mm) | | | |
|---|---|---|---|---|
| | *Enterobacter* sp. | *Staphylococcus* sp. | *Proteus* sp. | *Klebsiella* sp. |
| *E.coli* | 14.33±0.30 | 10.63±0.20 | 12.46±0.32 | 10.52±0.26 |
| *Pseudomonas* sp. | 16.66±0.05 | 15.30±0.10 | 24.76±0.15 | 24.70±0.10 |
| *Actinomycetes* sp. | 8.73±0.05 | 8.83±0.11 | 14.26±0.25 | 15.31±0.52 |
| *Aspergillus* sp. | 22.30±0.52 | 10.50±0.10 | 14.30±0.26 | 15.30±0.20 |
| *Penicillium* sp. | 10.26±0.25 | 10.76±0.15 | 30.16±0.20 | 26.10±0.10 |

## 4. Conclusion

This work determines the new way of preparing microbial active extract with chemical approach ie., by applying nanotechnology. The antimicrobial activity of silver nanoparticles showed the concentration dependent activity. It gives activity against all the test organisms. The active nanocompound from these organisms can be prepared and used effectively for preventing the growth of the bacterial pathogens. Among the three bacterial and two fungal species, the silver nanoparticles from the fungal organisms showed the better result against pathogens.

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