Synthesis and antibacterial activity of of silver nanoparticles

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Abstract. Silver nanoparticles have been known to have inhibitory and bactericidal effects but the antimicrobial mechanism have not been clearly revealed. Here, we report on the synthesis of metallic nanoparticles of silver using wild strains of Penicillium isolated from environment. Kinetics of the formation of nanosilver was monitored using the UV-Vis. TEM micrographs showed the formation of silver nanoparticles in the range 10-100 nm. Obtained Ag nanoparticles were evaluated for their antimicrobial activity against the gram-positive and gram-negative bacteria. As results, Bacillus cereus, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa were effectively inhibited. Nanosilver is a promising candidate for development of future antibacterial therapies because of its wide spectrum of activity.

1. Introduction
Nanotechnology is a most promising field for a generation of new applications in medicine. However, only few nanoproducts are currently in use for medical purposes. A most prominent nanoproduct is nanosilver. Ag-nanoparticles have been synthesized through an array of methods, e.g. spark discharging, electrochemical reduction, solution irradiating and cryochemical synthesis. At nanoscale, silver exhibits remarkably unusual physical, chemical and biological properties [1, 2].

With the increase of microbial resistance to multiple antibiotics many researchers have tried to develop new, effective antimicrobial agents free of resistance and cost. It is well known that Ag ions and Ag-based compounds are highly toxic to microorganisms, showing strong biocidal effects on as many as 12 species of bacteria including E.coli [3]. For a long time silver has been used to control bacterial growth in a variety of application including dental work and burn wounds [4, 5]. The antimicrobial mechanisms of silver may include modifications of sulfhydryl-containing biomolecules such as proteins, the collapse of electrochemical gradients across the bacterial cell membranes and the generation of reactive oxygen species [6].

Silver nanoparticles have also inhibitory and bactericidal effects [7]. It can be expected that the large specific area and high fraction of surface atoms of silver nanoparticles will led to high antimicrobial activity as compared with bulk silver metal [8]. Furthermore nanosilver can be modified for better efficiency to facilitate its application.

In this study we report the synthesis of silver nanoparticles by two Penicillium strains (K1 and K10) isolated from soil. Additionally these nanoparticles were evaluated for antimicrobial activity against gram-positive and gram-negative bacteria. The results suggest that Ag nanoparticles can be used as effective growth inhibitors in various microorganisms.
2. Materials and Methods

2.1. Synthesis of silver nanoparticles
Two *Penicillium* strains (denoted K1 and K10), isolated from the soil have been used in the study. Inoculated fungi were prepared in Petri dishes at room temperature using 2% malt extract with 0.5% yeast extract. Fungal biomass used for biosynthetic experiments was grown aerobically in liquid medium containing (g/l): KH$_2$PO$_4$ 7.0; K$_2$HPO$_4$ 2.0; MgSO$_4$×7H$_2$O 0.1; (NH$_4$)$_2$SO$_4$ 1.0; yeast extract 0.6; glucose 10.0. The Erlenmeyer flasks were inoculated with spores and incubated at 25°C with shaking (150 rpm) for 72 h. After the incubation, the biomass was filtered (Whatman filter paper No. 1) and then extensively washed with distilled water to remove any medium component. Fresh and clean biomass was taken into the Erlenmeyer flasks, containing 100 ml of Milli-Q deionised water. The flasks were agitated at the same conditions as described above, then the biomass was filtered again (Whatman filter paper No. 1) and cell-free filtrate was used in next experiments. AgNO$_3$ (1 mM of final concentration) was mixed with cell-free filtrate in an Erlenmeyer flask and agitated at 25°C in dark. The control (without the silver ions) was also run along with the experimental flasks.

2.2. Characterization of silver nanoparticles
To verify reduction of silver ions the solution was scanned in the range of 200-800 nm in a spectrophotometer (HELIOS λ, ThermoElectron Corp.). The size and morphology of the nanoparticles were analysed with the transmission electron microscope (JEOL). The sample was prepared by placing a drop of silver nanoparticles on a carbon-coated copper grid and subsequently drying in air before transferring it to the microscope.

2.3. Analysis of the antibacterial activity of silver nanoparticles
The silver nanoparticles were tested for their antibacterial activity by the agar diffusion method. Gram-positive bacteria represented by *Staphylococcus aureus* and *Bacillus cereus*, gram-negative bacteria represented by *Escherichia coli*, *Serratia marcescens* and *Pseudomonas aeruginosa* were used for this analysis. These bacteria were seeded in agar plates by the pour plate technique. Four cavities were made using a cork borer (7 mm diameter) at an equal distance and were filled with the silver nanoparticles solution (100 µL) and then incubated at 28°C or 37°C. The formation of a clear zone (restricted bacterial growth) around the cavity is an indication of antibacterial activity.

3. Results and Discussion
The silver nanoparticles were synthesized in the extracellular cell-free filtrates of the filamentous fungi K1 and K10. The appearance of a brown colour in the reaction vessels suggested the formation of nanoparticles (figures 1 and 2).

![Figure 1](image1.png)

**Figure 1.** Cell filtrate of *Penicillium sp*. K1 strain with silver ions: a) at the beginning of the reaction, b) after 72 h of the reaction
Figure 2. Cell filtrate of Penicillium sp. K10 strain with silver ions: a) at the beginning of the reaction, b) after 72 h of the reaction

The nanoparticles were characterized by the UV-Vis spectroscopy. The absorption spectrum (figures 3 and 4) of Ag nanoparticles prepared by biological reduction showed a surface plasmon absorption band with a maximum of about 400 nm, a characteristic peak of silver nanoparticles [9], indicating the presence of Ag nanoparticles in the solution. Due to the excitation of plasma resonance or interband transition, some metallic nanoparticles dispersion exhibits unique band/peaks [10]. The broadness of the peak is the good indicator of the size of nanoparticles. As the particle size increases, the peak becomes narrower with a decreased bandwidth and an increased band intensity [11].

Figure 3. Growth of UV-Vis absorption for Penicillium sp. K1 during the incubation time (0 to 168 h)

The absorption maximum of the studied nanoparticles were found to be 425 nm for the strain K10 (figure 3) and 422 nm for the strain K1 (figure 4), corresponding to the surface plasmon resonance of silver nanoparticles.
It is reported that the absorption spectrum of spherical silver nanoparticles presents a maximum between 420 nm and 450 nm with a blue or red shift when particle size diminishes or increases, respectively. For this reason, the nanoparticles produced by tested fungi present a plasmon, which is red shifted.

Apart from this, absorption peaks were observed in the UV region corresponding to 220 nm and 280 nm. While the peak at 220 nm may be due to the amide band, the other peak at 280 nm may be attributed to the tryptophan and tyrosine residues present in the protein that might have stabilized the nanoparticles [12, 13].

Transmission electron microscopy has provided further insight into the morphology and size details of studied nanoparticles. Figures 5 and 6 show representative TEM images recorded from drop-coated films of the silver nanoparticles synthesized by treating silver nitrite solution with cell-free filtrates of tested molds for 72 h. In general the particles are nanosized and well dispersed. The silver nanoparticles formed were predominantly spherical and polydisperse with diameters in the range of 10 nm to 100 nm for the K1 strain and 18 nm to 100 nm for the K10 strain.

Under careful observation it is noted that the silver nanoparticles are surrounded by a thin layer of other materials which we suppose is the capping organic material from the molds. It suggests that the biological molecules could possibly perform the function for the stabilization of the silver nanoparticles.

**Figure 4.** Growth of UV-Vis absorption for *Penicillium sp.* K10 during the incubation time (0 to 43 days)
Figure 5. TEM images of silver nanoparticles synthesized by reduction of silver ions using cell-free extract of K1 strain (x 215000)

Figure 6. TEM images of silver nanoparticles synthesized by reduction of silver ions using cell-free extract of K10 strain (x 130000).

The antibacterial activity of silver species has been well known since the ancient times [4, 5]. Moreover, it has been demonstrated that, in low concentrations, silver is non toxic to human cells [14, 15].

The studied silver nanoparticles exhibited excellent antibacterial activity against bacteria, *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Figures 7 and 8 show the clearing zones around the holes with bacteria growth.

Figure 7. Silver nanoparticles (K1 and K10) show the zone of inhibition against *Pseudomonas aeruginosa*

Figure 8. Silver nanoparticles (K1 and K10) show the zone of inhibition against *Staphylococcus aureus*

The diameter of inhibition zones (in millimetres) around the nanosilver against test strains are shown in table 1.

| Table 1. Zone of inhibition (mm) of nanoparticles against bacteria tested. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | *S. aureus*     | *B. cereus*     | *E. coli*       | *S. marcescens* | *P. aeruginosa* |
| **Penicillium sp.** |                 |                 |                 |                 |                 |
| K1               | 15              | 13              | 14              | -               | 16              |
| **Penicillium sp.** |                 |                 |                 |                 |                 |
| K10              | 14              | 12              | 14              | -               | 15              |

The studied nanosilvers were completely inactive toward *Serratia marcescens* but inhibited the growth of residual gram-negative rods *E.coli* and *P. aeruginosa*. The most sensitive was the
**Pseudomonas aeruginosa** strain. Moreover, the tested silver nanoparticles were active against the gram-positive bacteria *S. aureus* and *B. cereus*, which follows from the data collected in table 1. Particularly sensitive was coccus.

The actual bactericide mechanism of silver nanoparticles is not clear. Several studies have investigated the interaction of the nano-Ag with bacteria. Sondi and Salopek [16] and Morones et al. [17] revealed that the majority of the nanosilvers were localized on the membranes of treated *E.coli* cells. Xu et al [18] demonstrated the association of Ag nanoparticles to *Pseudomonas aeruginosa* when added at picomolar levels. Lok et al. [19] demonstrated that treatment of *E.coli* cells with nanomolar concentration of nano-Ag results in the immediate dissipation of the proton motive force, killing the cells. Such a biological mode of action is similar to that found for the silver nitrate.

4. Conclusion

In conclusion, we have reported the simple biological way for synthesizing the silver nanoparticles. The kinetics of extracellular synthesis of Ag-nanoparticles using a cell-free filtrate of two *Penicillium* strains is presented. The synthesis process was quite fast and nanoparticles were formed within hours of silver ion coming in contact with the cell filtrate. The results of TEM suggested that the protein might have played an important role in the stabilization of silver nanoparticles. The nanosilver were found to have wider antimicrobial activity than those described earlier reports [20, 21]. However, further studies must be done to verify if the bacteria develop resistance toward the nanoparticles and examine cytotoxicity of nanosilver towards human cells.

This process of the nanoparticles production is eco-friendly as it is free from any solvent or toxic chemicals, also easily amenable on a large-scale production.

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