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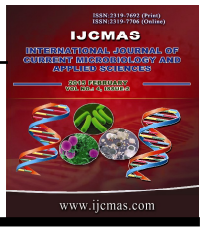

Original Research Article

Biosynthesis and Size of Silver Nanoparticles Using *Aspergillus niger* ATCC 16404 as antibacterial activity

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Keywords

Silver nanoparticles, *Aspergillus niger*, Antibacterial and TEM

A B S T R A C T

In this study, the biosynthesis of silver nanoparticles using of *Aspergillus niger* ATCC 16404 was produced silver nanoparticales. Predominantly monodispersed and spherical silver nanoparticles (AgNPs) in the size range of 11.19- 36.83 nm upon addition of 0.3 mM silver nitrate. The AgNPs were characterized by determining morphology and shape of nanoparticles.

Introduction

Nanoparticles are used nowadays in many fields of sciences such as chemistry, physics, medicine and biology. Nanotechnologies can solve a numerous problems. Metal-based nanoparticles are derived from silver, iron, titanium and a variety of other metals and their oxides pal et al.,(2007). Biosynthetic and environment friendly technology for the synthesis of silver nanoparticles (silver NPs) are believed to be nontoxic, biosafe, and biocompatible and have been used as antimicrobial and fillings in medical materials . a number of physical and chemical strategies were employed for the synthesis of AgNPs Inoue et al.,2010. However, concern has been raised on the toxicity of chemical agents used in AgNPs synthesis. Thus, it is essential to develop a green approach for AgNPs production without using hazardous substances to the human health and environment. Compared with the traditional synthetic methods, biological systems provide a novel idea for the production of nano-materials Bansal et al., (2011). Up to now, several microorganisms from bacteria to fungi have been reported to synthesize inorganic materials either intra- or extracellularly, and thus to be potentially utilized as eco-friendly nanofactories Mohanpuria, et al.,(2008).

Materials and Methods

Materials

Aspergillus niger ATCC 16404 maintained on potato dextrose agar (PDA) medium at 28°C. Potato dextrose broth and MRS media

were purchased from Sigma. The chemical silver nitrate ($AgNO_3$) was purchased from Sigma-Aldrich.

Biomass preparation

Aspergillus niger was grown in MRS at 28°C on a rotary shaker (120 rpm) for 96 h. The biomass was harvested by filtration using Whatman filter paper No. 1, followed by washing with distilled water to remove any components of the medium.The biomass (15 gm) wet weight was placed in individual flasks containing 100 mL water and incubated as described above for 96 h. The biomass was filtered, and the cell filtrate was collected and used for biosynthesis of AgNPs.

Biosynthesis of AgNPs

For biosynthesis of AgNPs, 50 ml of cell filtrate was mixed with 0.3M $AgNO_3$ solution and reaction mixture without $AgNO_3$ was used as control. The prepared solutions were incubated at 28 °C for 24 h. All solutions were kept in dark to avoid any photochemical reactions during the experiment. The AgNPs were purified by centrifugation at 10,000 rpm for 10 min twice, and collected for further characterization Sagar and Ashok, (2012).

Characterizations of AgNPs

Transmission electron microscopy (TEM)

TEM analysis, a drop of aqueous solution containing AgNPs were placed on the carbon coated copper grids and dried by allowing water to evaporate at room temperature. Micrographs were obtained using operating at 200 kV. The sizes of AgNPs were estimated by determine the width of reflection Rai et al., 2009 and size distribution of the resulting nanoparticles was also estimated on the basis of TEM micrographs.

The Antimicrobial Activity Analysis of AgNPs

The antimicrobial activity of AgNPs was investigated against *P. aeruginosa ATCC 9027, E. coli ATCC 8739, B. Subtilis 6633 and S. typhimurium14028* using disk diffusion assay. Each strain was swabbed uniformly onto individual plates after incubation at 28 °C for 28 h, the diameter of inhibition zone. The assays were performed in triplicate Mehdi et al., (2014).

Result and Discussion

Synthesis and Characterization of AgNPs Using *Aspergillus niger*

In this study**,** AgNPs were synthesized using a reduction of aqueous Ag^+ with the culture supernatants of *Aspergillus niger* ATCC 16404 at room temperature. It was generally recognized that AgNPs produced brown solution in water, due to the surface plasmon resonances (SPR) effect and reduction of $AgNO_3$ Bansal et al.,(2010). After the addition of $AgNO_3$ solution, the cell filtrate of A. niger changed from light yellow to brown in a few hours, while no color change was observed in the culture supernatant without $AgNO_3$(Figure 1).

Thus, color change of the solution clearly indicated the formation of AgNPs. The color intensity of the cell filtrate with $AgNO_3$ was changed after 24 h incubation, which indicated that the particles were well dispersed in the solution, and there was no obvious aggregation. All these reactions were monitored by ultraviolet-visible spectroscopy of the colloidal AgNPs solutions.The ultraviolet-visible spectra of the cell filtrate with $AgNO_3$ showed a strong

broad peak at 440 nm which is surface Plasmon resonances (SPR band), which indicated the presence of AgNPs. These results were consistent with the reports of Verma et al.,(2010). The intensity of the SPR band steadily increased from 6 h to 24 h as a function of time of reaction. It was also observed that the AgNPs formed were quite stable in the supernatant of A. niger. The application of AgNPs was highly dependent on the chemical composition, shape, size, and monodispersity of particles Pal et al.,(2007). To broaden the application scope, the AgNPs obtained were systematically characterized using TEM. Through the TEM analysis, the particles were spherical and poly disperse with an average size of 4.3 nm (1–20 nm), and the majority of the particles were less than 10 nm (Figure 3).

TEM measurements were used to determine the morphology and shape of nanoparticles. TEM micrographs (Fig. 3) revealed that the particles are spherical in shape and uniformly distributed (monodispersed) without significant agglomeration. The particle size of silver nanoparticles shows that the particle size ranges from 5 to 35 nm. These results are compatible with ki-young,2011. Very tiny particles were appeared (smaller than 40 nm) that may be due to vigorous shaking Bansal et al.,(2010). The highest number of particles distribution observed the particles are in the 11.19 to 36.83-nm range.

Antimicrobial Activity Analysis of AgNPs

The antimicrobial activity of AgNPs against various pathogenic organisms including bacteria was investigated. Compared with the control, the diameters of inhibition zones increased for all the test pathogens (Table 1). The AgNPs produced could inhibit three different typical pathogenic bacteria, including *Salmonella typhimurium* ATCC 14028, *Bacillus Subtilis* ATCC 6633, Pseudomonas aeruginosa ATCC 9027 and *Escherichia coli* ATCC 8739, as previously described Bansal et al.,(2010). Thus, AgNPs could be considered as excellent broad-spectrum antibacterial agents. Which were the most important pathogenic. Since the biosynthesized AgNPs showed considerable antimicrobial activity, they could be potential to be widely used in clinical applications.

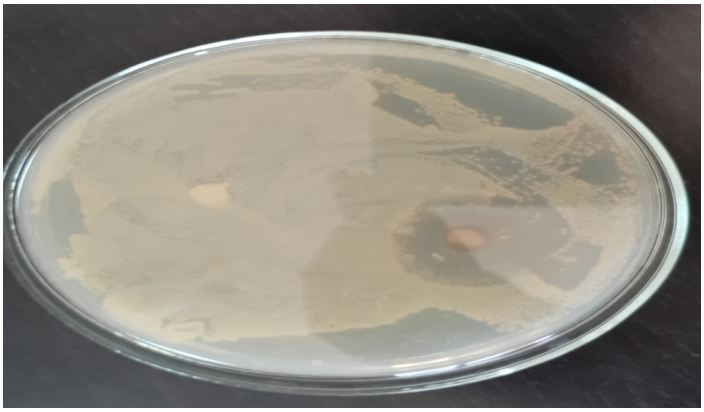
Antibacterial activity of silver nanoparticles has been demonstrated in several investigations. In the present study, silver nanoparticles showed good antibacterial activity against all the tested pathogens. In the present study, silver nanoparticles showed good antibacterial activity against all the tested pathogens. This may be due to the differences in bacterial cell walls, since Gram negative bacteria have thinner cell wall comparing to Gram positive bacteria Birla et al.,(2009).In agreement, Kim et al.,(2007) reported that *E. coli* ATCC 8739 was more resistant against nanosilver than Gram postive *Pseudomonas aeruginosa* ATCC 9027 Rai et al.,(2009). It has dependented on the concentration and size of nanoparticles and also the initial bacterial concentration Wang et al.,(2009). Silver nanoparticles with size of 11.19-36.83 nm have been reported to be most effective against bacteria through direct interaction with bacterial cells kim et al.,2009 that found the interaction of nanoparticles with *Pseudomonas aeruginosa* ATCC 9027 was shape-dependent particles showed the highest activity. Silver nanoparticles showed great antibacterial effects on four important foodborne pathogens. Therefore, with development of multidrug-resistant strains of bacteria, Ag NPs could be good alternatives for cleaning and disinfection of equipment and surfaces in food-related environments.

Table.1 Antibacterial activity of silver nanoparticles

	***Salmonella typhimurium* ATCC 14028**	***Bacillus Subtilis* ATCC 6633**	***Pseudomonas aeruginosa* ATCC 9027**	***Escherichia Coli* ATCC 8739**
Silver Nitrate	0.0	0.0	0.0	0.0
Silver Nanoparticles	2.2	2.5	3.4	2

Fig.2 Antimicrobial activity of silver nanoparticles against bacterial pathogens used in the Experiment (A) Pseudomonas aeruginosa 9027 (B) Bacillus Subtilis 6633

(A) ***Psedumonas aeruginosa* 9027**



(B) *Bacillus Subtilis* 6633

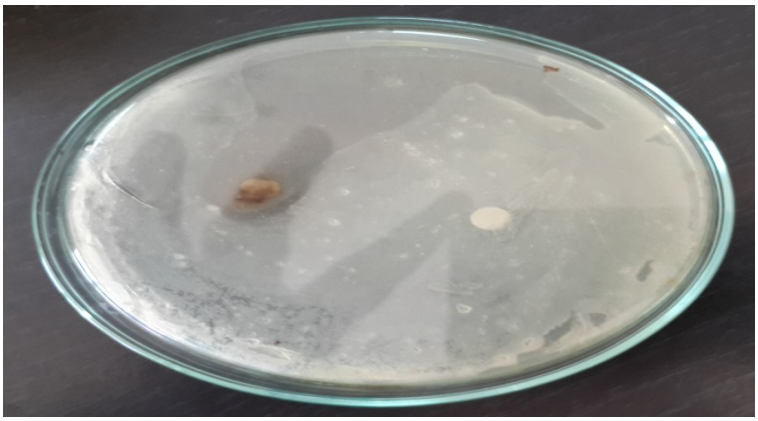
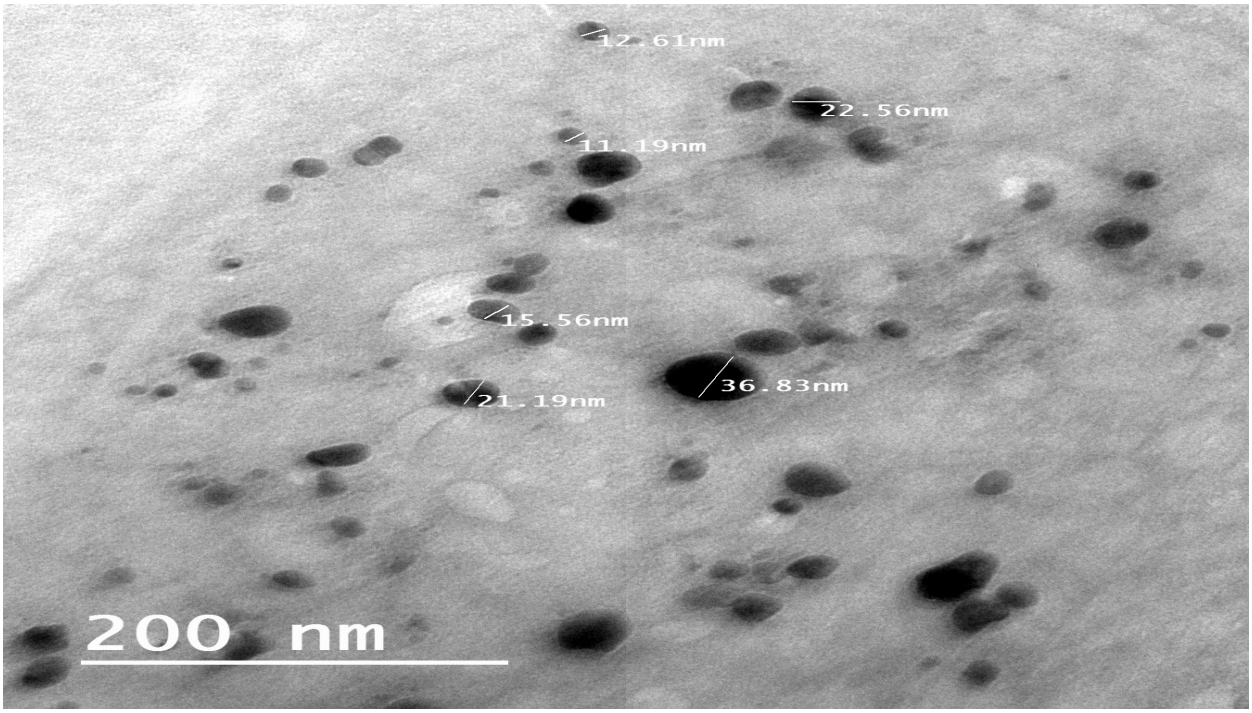


Fig.3 Field Transmission electron microscope (TEM) images with of Ag nanoparticles



The genus *Chryseobacterium* belongs to the family *Flavobacteriaceae*. Six species of *Chryseobacterium* are commonly isolated from clinical specimens: *C. meningosepticum*, *C. odoratum*, *C. multivorum*, *C.breve* and group IIb *Chryseobacterium* species, which includes *C. indologenes* and *C. gleum. Members of genus Chryseobacterium* are Gram-negative, aerobic, nonfermentative, oxidase and catalase-positive, non-motile bacilli that produce a distinct yellow to orange pigment. They are readily distinguished from other non-fermenters by their ability to produce indole in tryptophan broth, but the reaction often is weak and difficult to demonstrate. [7] *C. indologenes* is an uncommon human pathogen. The clinical significance of *C. indologenes* has not yet been fully established, because this bacterium has not been frequently recovered from clinical specimens. Reported infections include bacteremia, ventilator-associated pneumonia, indwelling device associated infection, urinary tract infections, biliary tract infection, peritonitis, lumboperitoneal shunt infection, ocular infections, surgical and burn wound infections. [8] In present case patient underwent catheterization and instrumentation for artificial rupture of membrane.

C. indologenes is a rare human pathogen reported to have caused hospital-acquired infections in Taiwan and rarely elsewhere. Six cases have been reported in Europe, two in Australia, and four in the USA. In addition, ophthalmic *C. indologenes* infections have been reported including one case report in the USA, one in Taiwan and one in Spain. [9]

Majority of studies from India have reported *C. meningosepticum*. Recently however, Sudharani *et al* have reported *C. indologenes* bacteremia in a preterm baby, where environmental sampling did not yield the source of infection [10] and Eshwara VK

et al reported neonatal meningitis and sepsis due to *C. indologenes.* [11]

Antimicrobial susceptibility data on *Chryseobacterium* species remain very limited. In addition, results of susceptibility testing vary when different methods are used. [12]

Results from disk diffusion methods may not be reliable, so broth reference quality microdilution tests should be performed when possible. According to the results of the SENTRY Antimicrobial Surveillance Program (1997-2001), the most active agents against *C. indologenes* are the quinolones (garenoxacin, gatifloxacin, and levofloxacin) and trimethoprim-sulfamethoxazole (≥95% susceptibility), followed by piperacillin-tazobactam (90% susceptibility). Ciprofloxacin, cefepime, ceftazidime, piperacillin, and rifampin are showing reasonable activity (85% susceptibility). On the contrary, aminoglycosides, other *β-lactams*, chloramphenicol, and linezolid are not appropriate for treating infections by this organism. [13] The present isolate demonstrated susceptibility to tetracycline, tigecycline and trimethoprim-sulfamethaxazole but not to quinolones, betalctams and aminoglycosides. The Vitek system also revealed the similar results with sensitivity to minocycline, tigecycline, and trimethoprim-sulfamethaxazole but not to quinolones, betalctams and aminoglycosides.

In conclusion, since *C. indologenes* is a betalactamase producer and demonstrates high level of multi drug resistance with inherent resistance to carbapenems, empirical antibiotic therapy may not cover this organism. Proper management of infection by this relatively resistant organism warrants correct identification and antimicrobial susceptibility testing.

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