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Fabrication of silver nanoparticles by Phoma glomerata and its combined effect against Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus

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Abstract

Using Phoma glomerata, we synthesised extracellular silver nanoparticles (Ag-NPs) and tested their efficiency against Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa. A combination of antibiotics and Ag-NPs exhibited extraordinary sensitivity to bacteria that were resistant to other anti biotics. Methods and Results: The silver nitrate (1 mmol l)1 was used to challenge the fungal cell filtrate in order to biosynthesise Ag-NPs. Characterization of the Ag-NPs was carried out using a UV–Visible spectrophotometer and a Fourier transform infrared spectrometer. The size of Ag-NPs was determined using scanning electron microscopy. Evaluation of the combined effect(s) against E. coli, Staph. aureus, and Ps. aeruginosa was done using the disc diffusion technique. In the end, the biosynthetic technique seems to be environmentally benign and simple to scale up the process. Because of their biogenic character, these Ag-NPs may show to be a superior medication candidate and avoid the issue of chemical agents. The Study's Importance and Impact: Antibiotic-resistant microorganisms are emerging at an alarming pace. An urgent need exists for the development of bactericides to address this issue effectively. Bacteria that are resistant to antibiotics may be able to be treated using Ag-NPs.

Keywords

Drug resistance, extracellular, Fourier transform infrared spectroscopy, Phoma glomerata, scanning electron microscopy, and silver nanoparticles are some of the other terms associated with this study.

Introduction

Engineering functioning systems at the molecular scale is what nanotechnology (NT) is all about. An NT is the capacity to operate at the molecular, atomic, and supramolecular levels in order to comprehend, build, and utilise materials, technologies, and systems that have fundamentally different characteristics and functionalities because of their tiny size. In order to investigate and modify biological systems, NT uses tools and technological platforms provided by biology, while biology gives NT with inspiration models and bio-assembled components (Mihail 2003). Topical bactericides such as silver are used in medical practise (Yamanaka et al. 2005). Nanoparticles have more surface atoms than microparticles, which considerably enhances their physical and chemical properties as a result of the advancement of NT. Even though physical and chemical techniques exist to synthesise silver nanoparticles (Ag-NP), they need a great deal of energy to sustain high pressure and temperature. Humans may be harmed by the use of dangerous substances during the synthesis process (Chen et al. 2003).

It is vital to note that micro-organisms such as bacteria and fungus may play a key role in the remediation of hazardous metals, which is why these biological systems have been widely exploited for the quick and environmentally benign manufacture of metal nanoparticles (Bhattacharya and Gupta 2005). Nanoparticles may be synthesised both intracellularly and extracellularly by uni- and multicellular organisms, since they can produce inorganic materials both in and out of the cell.

Studies on the use of microbes in the synthesis of various metal nanoparticles have been conducted in the recent past, including studies on bacteria for gold (Beveridge and Murray 1980); silver (Joerger et al. 2000); CdS (Smith 1998); ZnS (Labrenz 2000); magnetite (Lovley et al. 1987); iron sul fide (Watson et al. 2001); and iron sul fide sulphate (Robinson et al. 1997). Fungi have been used extensively to synthesise a variety of different metal nanoparticles, including gold nano particles from Fusarium oxysporum and Colletotri chum species, thermomonospora sp., and Lichen fungi (Usnea longissima) and silver nanoparticles from Fusarium acu minatum, F. semitectum, and Aspergillus f. Among other things, these fungi have also been used to synthesise silver nanoparticles from F. acu minatum and Aspergillus f. (Bansal et al. 2004). When Candida glabrata is exposed to Cd2+ ions, CdS

quantum dots develop within the cell (Dameron et al. 1989). Although bacteria are easier to cultivate, fungi have a number of benefits over them, including the ability to synthesise most of their molecules extracellularly, which makes them an excellent candidate for downstream processing and the ability to handle Ag-NPs more easily. The mycelia of a fungus may grow to a reasonable size and cover a vast surface area with ease, making the system economically viable. A number of antibacterial applications for Ag-NPs are previously known (Sondi and Salopek-Sondi 2004; Kim et al. 2007; Shrivastava et al. 2007). Using cloths infused with Ag-NP reduces the spread of harmful microorganisms like Staphylococcus aureus in hospitals (Duran et al. 2007). Extracellular mycosynthesis of Ag NPs by A. niger and F. acuminatum was reported by Gade et al. (2008) and
Ingle et al. (2008), respectively. Staph. aureus, Salmonella typhi, Staph. epidermis, and E. coli were among the harmful and multidrug resistant bacteria that they were able to kill with this treatment. We employed Phoma glomerata, a common plant pathogen, to synthesise Ag-NPs in this investigation since it is cosmopolitan in nature and widely distributed.

Phoma species and materials

Ag-NPs were synthesised using Phoma glomerata (MTCC-2210). Microbial Type Culture Collection Center (MTCC), IMTECH, Chandi garh provided the culture. The culture was maintained on potato dextrose agar (250 g potato infusion, 20 g dextrose, 20 g agar, and 1 l distilled water) and kept at 4 C for future use.

Using Phoma glomerata, the synthesis of Ag-NPs.

There were two Erlenmeyer flasks, each with 100 ml of potato dextrose broth, in which the fungal mycelium was cultivated. They were kept at a temperature of 25 2 C (Borosil, India) for 48 hours and 120 revolutions per minute. Water treated with distilled disinfectant was used to wash Mycelia through Whatman filter paper number 42. Next, 100 ml of sterilised distilled water with mycelium suspended in it was incubated at 25 2 degrees Celsius for 24 hours. Filtration was used to remove the cell debris, which was then incubated at room temperature with silver nitrate solution. Measurement of absorptance was done using a UV– Visible spectrophotometer on the cell filtrate that had been treated. Fungal cell filtrate with a dark brown colour suggests the presence of Ag-NPs.

In the case of Ag-NPs, the detection

Spectroscopy in the range of ultraviolet and visible light When silver nitrate was added to cell filtrate, the colour of the filtrate changed significantly. A UV– Visible spectrophotometer (Lambda 25; PerkinElmer) was used to further characterise the produced Ag-NPs by scanning the absorbance spectra in the range of 250–800 nm.

Ag-NPs Characterization

Infrared spectroscopy using Fourier transforms. Centrifugation at 10 000 g for 15 minutes was performed on the fungal filtrate after it had been reduced to Ag+ ions, and the supernatant was replaced with distilled water each time. When sodium chloride is added to silver ions that haven't been reacted, a white precipitate forms. However, no precipitate was produced when sodium chloride was added to the nanoparticle solution, demonstrating the lack of unreacted silver. Fou rier transform infrared spectroscopy (FTIR) was used to analyse the sample in the range of 450–4000 cm)1 at a resolution of 4 cm1 for the FTIR spectrum.

SEM, or scanning electron microscopy,

A JEOL 6380A scanning electron microscope was used to get the images. Glutaraldehyde was used to fix the samples overnight at room temperature. In order to synthesise Ag-NPs, the fixed sample was dehydrated in a gradient alcohol (10–95 percent) and then incubated for 20 minutes in each gradient. The sample was then dipped in Phoma glomerata. Absolute alcohol was used for 2–5 minutes by the Authors. In order to make the surface of the specimen conduct, a drop of dehydrated material was placed on a glass slide and then coated with a monolayer of platinum. **Ag-NPs have antibacterial properties.**

On Muller–Hinton agar plates, several antibiotics were tested for bactericidal activity using the disc diffusion technique against the test microorganisms E. coli JM103 (ATCC-

39403), Staph. aureus (ATCC-25923), and Ps. aeruginosa (MTCC 424). (Bauer et al. 1966). The bacterial cultures were kept at 4 degrees Celsius on nutrient agar slants. HiMedia Laboratories Pvt. Ltd. supplied the usual antibiotic CDs (Mumbai, Maharashtra, India). Each standard paper disc was coated with 15 ll of solution of Ag-NPs in order to examine their combined effects. Ag-NPs alone were used in similar experiments. Overnight, one colony of each strain was cultivated in Muller–Hinton liquid medium at 37 C on a rotary shaker (100 revolutions per minute)2. Finally, the inocula and produced discs containing Ag-NPs were put to the plates. The inhibitory zone was evaluated after 24 hours of incubation at 37 C. The tests were run three times each.

To determine how much fold area has grown

It was determined by estimating the average surface area of the inhibitory zone for each tested antibiotic. Using the formula (b2) a2) a2, the area of Staph. aureus's inhibition zone (A) and the area of Staph. aureus+Ag-inhibition NPs's zone (B) were computed. A similar equation was used to calculate E. coli's fold increase area using the equation (d2) c 2)c 2, where C and D represent zones of inhibition for antibiotic and antibiotic + Ag-NPs, respectively; for Ps. aeruginosa, the equation (f 2) e 2)e 2, where E and F represent zones of inhibition for antibiotic and antibiotic + AgNPs, respectively, was used to calculate the fold increase area.

Results

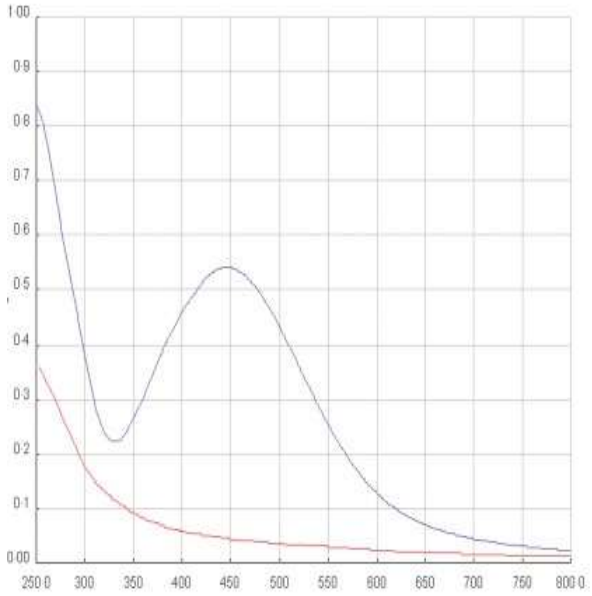
After treatment with AgNO3 (1 mmol l)1 in the reaction vessels, the cell filtrate became dark brown from light yellow, indicating the synthesis of Ag-NPs. UV–Vis spectroscopy was used to characterise the AgNPs. The distinctive plasmon reso nance, which revealed an absorbance peak at 440 nm in the UV–Vis spectrum, provides confirmation of the existence of noble Ag-NPs by extinguishment spectroscopy (UV– Vis spectrum) (Fig. 2). Analysis of FTIR (PerkinElmer) data was used to determine the probable biomolecules that might be responsible for the phenomenon.x

Silver nitrate (1 mmol l)1 in conical flasks before (left) and after (right) exposure to Phoma glom erata culture supernatant

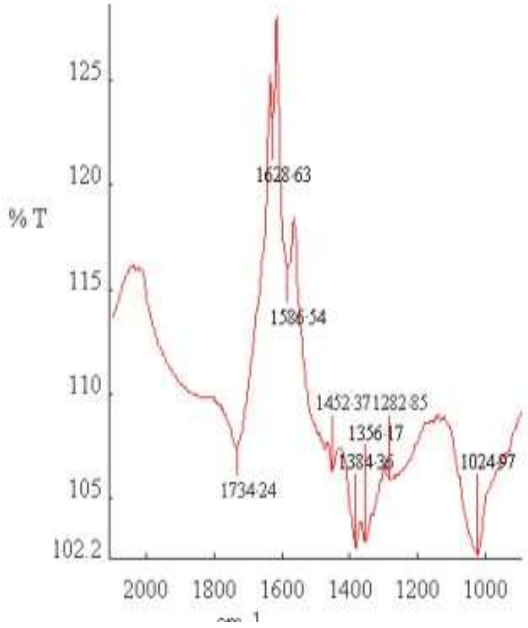
decrease Ag+ ions and cap bioreduced Ag-NPs generated by fungal filtrate to avoid agglom eration of particles and medium stabilisation (Basavaraja et al. 2008). For example, functional groups were found in the region of 1000–2000 cm)1 in the FTIR spectrum, which indicates the existence of stabilising protein molecules (Fig. 3). According to results shown in Figure 4 from scans using a SEM microscope, the reaction mixture had really been synthesised to produce round Ag-NPs. An average Ag-NP size of 60– 80 nm was discovered in the produced materials. The combined impact of Ag-NPs against three harmful bacteria, Staph. aureus, E. coli, and Ps. aeruginosa, was examined using the disc diffusion technique in contrast to commercially available antibiotics. Table 1 displays the inhibition zone sizes (measured in millimetres) surrounding several antibiotics, both with and without Ag-NPs, against various test bacteria. Antibacterial activity of ampicillin, gentamycin, streptomycin, and vancomycin against Gram-negative bacteria, such as E. coli and Ps. aeruginosa, was boosted when these drugs were combined with AgNPs as opposed to Staph. Ampicillin, streptomycin, and vancomycin all showed significant fold increases in surface area. For this reason, compared to Staph. Aureus, synergistic action was better in E. coli and Ps. aeruginosa

Discussion

Figure 1: Conical flasks containing silver nitrate (1 mmol l)1 before (left) and after (right) exposure to culture supernatant of Phoma glomerata, a pathogenic bacterium known to produce antibiotic resistance.
Phoma glomerata for the production of Ag-NPs by S.S. Birla et al.



UV–Vis spectrophotometer detection of silver nanoparticles in Phoma glomerata cell filtrate (where red represents fungal extract and blue represents fungal extract with 1 mmol l of silver nitrate) Figure 2.



Cm[1]

This is the FTIR spectrum of the extract of Phoma glomerata, after one mole of silver nitrate has been added to it.

The focus is on discovering new antibacterial agents. Ag-NPs are currently a serious challenger in the medical field as an antibacterial agent (Duran et al. 2007). Using nanoparticles instead of bulk materials has both benefits and downsides.

Fig. 4 depicts nanoparticles derived from the Phoma glomerata extract (Scale bar 0.05 lm).

Even yet, there's still a need to develop environmentally-friendly nanoparticle-production techniques (Chen et al. 2003).

Table 1 Zone of inhibition (mm) of different antibiotics against Staph. aureus, E. coli and Ps. aeruginosa [in absence and in presence of silver nanoparticles (Ag-NPs) at content of 15 µl per disc]

	Staphylococcus aureus			Escherichia coli			Pseudomonas aeruginosa		
Antibiotics	Antibiotic (A)	Antibiotic + Ag-NPs* (B)	Increase in fold areat	Antibiotic (C)	Antibiotic + Ag-NPs* (D)	Increase in fold areat	Antibiotic (E)	Antibiotic + Ag-NPs* (F)	Increase in fold areat
Ampicillin	18	20	0·234	_‡	10	1·777	_	11	2·361
Gentamycin	18	21	0·361	18	23	0·632	22	25	0·291
Kanamycin	22	25	0·291	20	25	0·562	16	17	0·128
Streptomycin	19	24	0·595	13	15	0·331	–	10	1·777
Vancomycin	17	22	0·674	_	11	2·361	_	11	2·361

At least 125 millimetres was the average diameter of the inhibition zone surrounding the Ag-NPs-only disc; Standard deviations were quite low since all studies were performed in triplicate. The mean diameter of each tested antibiotic was used to compute the mean inhibition zone surface area (mm2). A2 = (b2 - a2) a2 is the formula used to compute the fold increases for various drugs against Staph. aureus. Both (d2 - (f2 - (e2) - e2) - e2) were employed for antibiotics against E.coli and Ps.aeruginosa, respectively, in the same manner. To compute the fold increase in columns 3, 6, and 9 when the bacterial growth inhibition zones were not present, the disc's diameter (6 mm) was employed. Symbiosis of Ag with Phomaglomeratus pathway for synthesising Ag (Ingle et al. 2008). As a result, this research represents a significant step in the right direction. The colour of the fungal filtrate changed from light yellow to dark brown when silver nitrate (1 mmol l) was added in the present study of extracellular biosynthesis of AgNPs utilising P. glomerata1.

A. niger cell filtrate treated with silver nitrate (1 mmol l)1 developed a brown colour, which we found to be similar to the findings of Gade et al. (2008). Analysis of UV–Vis spectrophotometer data showed a sharp peak at around 440 nm, which was specific for the synthesis of Ag-NPs; these findings corroborate with the findings of Li et al., 2007; they observed that when Capsicum annum L. extract was challenged with aqueous silver ions, the reaction mixture with Ag-NPs showed the absorption peak at about 440nm because of the excitation of longitudinal silver. (Fig. 2) The amide connections between amino acid residues in proteins provide the well-known fingerprints in the infrared portion of the electromagnetic spectrum during the FTIR examination. There are absorption peaks in the band 1000–2000 cm (Fig. 3) that may be seen in the produced nanoparticles' spectra (see Fig. 3). C–O–C– or –C–O– are the most likely candidates for this peak, which is located at about 1025 cm)1 (Huang et al. 2007). In the FTIR spectrum, there was also a peak at roughly 1628 cm1 associated with the stretch vibration of –C=C– (Huang et al. 2007) and the amide I bonds of proteins (Sastry et al. 2003). There is a noticeable increase in the peak at around 1390 cm)1 suggesting the presence of NO3) in the solution (Luo et al. 2005).

The aromatic C–C skeleton vibrations are thought to be responsible for the 1595 cm)1 band (Sastry et al. 2003). We also reported the 1384 and 1586 cm)1 peaks in our study, which were similar to those found by Luo et al. (2005) and Sastry et al. (2006). (2003). If the peak at 1452 cm1) is due to symmetric stretching vibrations of –COO– groups of amino acid residues in the protein with free carboxylate groups, then this may be the case.2 3. (Shivshankar et al. 2004a,b) Capping ligands for nanoparticles are produced from heterocyclic chemicals and proteins found in the fungal extract, which include bonds or functional groups such as –C-O, -C-O, and –C=C–. (Sastry et al. 2003; Shivshankar et al. 2004a; Huang et al. 2007). There were spherical silver nanopartilces in the 60–80 nm size range seen in the SEM micrograph research, along with silver nanopartilces aggregates. These results support the findings

of Sadowski et al. (2008), who found that drying causes the nanoparticles to aggregate to some degree. Silver has shown to be the most effective nanosized antibacterial agent because of its ability to combat bacteria, viruses, and eukaryotic microorganisms (Feng et al. 2000; Morones et al. 2005). Ag-NPs were shown to have antibacterial action against E. coli by Sondi and Salopek-Sondi (2004) and were suggested for use in the development of novel bactericidal agents. Ag-NP antibacterial efficacy against E. coli and Staph. aureus was studied by Kim et al. (2007). While Sondi and Salopek-Sondi (2004) showed superior antibacterial activity against E. coli and Ps. aeruginosa, Shahverdi et al. (2007) observed better antibacterial activity against Staph. aureus in their study. In the meanwhile, further research is needed to determine whether or not these nanoparticles may be used as a more effective bactericide against additional Gram-positive and Gram-negative bacteria.

Conclusion

P. glomerata was used in this investigation to synthesise Ag-NPs. A simple biological procedure utilising P. glomerata has been described for the production of Ag-NPs. The FTIR study shows that AgNPs have been capped. Ag-NPs in a colloidal solution will be more stable because to the incorporation of these capped Ag-NPs. Drug delivery systems may find them more appealing since they are protected by biological substances. The produced Ag-NPs have broad bactericidal action against E. coli, Pseudomonas aeruginosa, and Staphylococcus aureus, among other pathogens. Antibiotics' antibacterial activity was boosted by Ag-NPs. Antibiotics and Ag-NPs show that antibiotic-resistant organisms are vulnerable to them. Because P.glomerata may be used to synthesise nanoparticles, the issue of chemical agents, which may have negative effects on the use of nanoparticles, might possibly be eliminated.

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